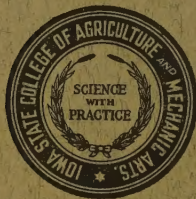


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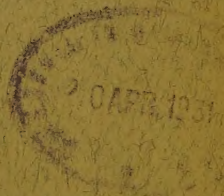
A Quarterly of Research



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PUBLISHED BY
THE IOWA STATE COLLEGE PRESS
PRESS BUILDING
AMES, IOWA



IOWA STATE COLLEGE JOURNAL OF SCIENCE

Published October, January, April, and July

EDITOR-IN-CHIEF	George F. Stewart
MANAGING EDITOR	Fred E. Ferguson
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Published under the joint auspices of the graduate faculty of Iowa State College and the local chapter of Sigma Xi. The administrative board for the academic year 1950-51 includes:

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All matter pertaining to subscriptions, remittance, etc., should be addressed to the Iowa State College Press, Press Building, Ames, Iowa. Subscriptions are as follows: Annual: \$6.00; (in Canada \$6.50; other foreign countries (\$7.00); single copies: \$2.00 (Except Vol. XXV, No. 2—\$3.00).

Entered as second-class matter January 16, 1935, at the post office at Ames, Iowa, under the act of March 3, 1879.

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CHEMISTRY OF GLYCEROL DICHLOROHYDRIN AS A REAGENT FOR THE DETERMINATION OF VITAMIN A¹

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The analysis of vitamin A by colorimetric methods has been the topic of many investigations in the past 25 years. Numerous methods have been proposed, tested, and finally discarded with the conclusion that they lacked specificity and/or the required sensitivity to measure small amounts of vitamin A in biological materials. In 1945 a new colorimetric reagent, which has many desirable features for the measurement of vitamin A, was introduced. The reagent is glycerol dichlorohydrin (GDH) which is activated by vacuum distillation with a small amount of antimony trichloride.

Pure glycerol dichlorohydrin gave no color test with vitamin A, but was activated by the addition of small amounts of certain inorganic acids, metallic and non-metallic halides, acyl halides and several other compounds. Compounds or solutions which were good activating agents included concentrated hydrochloric acid, concentrated sulfuric acid, chlorosulfonic acid, 60 per cent perchloric acid, phosphorus trichloride, phosphorus oxychloride, aluminum chloride, arsenic trichloride, and methyl sulfate. The hydrogen ion *per se* is not the sole activating agent since addition of nitric acid, acetic acid, monochloroacetic acid, or trichloroacetic acid did not result in an activated reagent.

Good activation of GDH was produced by concentrations of anhydrous hydrogen chloride ranging from 0.002 to 0.08 N, with optimum activity at 0.01 N hydrogen chloride.

Good activation was produced by concentrations of chlorosulfonic acid ranging from 0.02 to 0.5 per cent acid, with optimum activity at 0.1 per cent chlorosulfonic acid.

The activity of GDH activated with either anhydrous HCl or chlorosulfonic acid changed on standing in the laboratory. Reagents originally showing optimum activity became less active, while those having excess or mere traces of acid became somewhat more active on standing at room temperature and on exposure to laboratory light.

GDH activated by the addition of 1 per cent concentrated sulfuric acid produced a blue color when first mixed with a chloroform solution of vitamin A. Within 5 or 6 minutes the blue changed to a fairly stable but not very intense red-violet color with maximum absorption at 545 m μ and a smaller absorption band at 452 m μ .

¹ Doctoral thesis, number 1003, submitted October 5, 1949.

GDH was activated by heating up to 4 hours at 85°C. with concentrations of antimony trichloride ranging from 0.3 to 1 per cent. Activity was greatest with the higher levels and longer heating periods. The ultraviolet absorption spectrum of GDH activated by heating with 0.3 per cent antimony trichloride indicated a change in the reagent due to a reaction of antimony trichloride with GDH to produce a derivative containing antimony or a chemical bonding between the reactants.

Good activity was produced by vacuum distilling several brands of GDH with 1 per cent antimony trichloride. No significant difference between brands was observed when solvent reagent ratios of 1:5 and 5:1 were employed. The activity of these reagents was equal to or better than GDH activated with either HCl or ClSO_3H . Apparently antimony trichloride reacted with GDH during the heating and distillation to produce HCl along with a distillate which contained antimony.

Glycerol dichlorohydrin activated by the addition of concentrated hydrochloric acid, chlorosulfonic acid, or by vacuum distillation with antimony trichloride, when present in excess, reacted with vitamin A acetate to produce a violet color which had a principal absorption maximum at 555 m μ and a small absorption band at 358 m μ . The stability of the color was determined by measuring the absorption spectrum at various time intervals after initiation of the color reaction. In each case the extinction coefficient at the 555 m μ maximum decreased on standing.

Antimony trichloride-activated GDH was deactivated by heating with activated charcoal, filtering, addition of a small quantity of aqueous KOH and finally vacuum distilling to give a clear, colorless reagent. On standing in a clear, glass-stoppered bottle for several weeks, this product became active due to decomposition, a process which was hastened by heating. Free chloride ion was found in this active reagent. It was postulated that hydrogen chloride was one of the decomposition products responsible for the activation of the GDH.

Glycerol α -monochlorohydrin gave weak color tests when activated by the same agents which activated GDH. The absorption spectrum of the color produced when vitamin A reacted with antimony trichloride-activated glycerol monochlorohydrin was not unlike that of the GDH-vitamin A color except in magnitude. The monochlorohydrin color was only about half as intense as that produced by the GDH-vitamin A reaction when a 1:5 solvent-reagent ratio was used. At the 5:1 ratio the absorption at 373 m μ was nearly the same with both reagents. Vacuum distillation of 1,2,3-trichloropropane with 1 per cent antimony trichloride failed to produce an active reagent. It is concluded that no glycerol derivative thus far tested is as effective as a vitamin A colorimetric reagent as glycerol dichlorohydrin. This evidence supports the view that a hydroxyl group and two chlorine atoms, each on separate carbon atoms of a propane skeleton are required for maximum color formation with vitamin A.

Solvent-reagent ratios of 1:9, 1:7, 1:5, 1:3, 1:1, 3:1, 5:1, 7:1, and 9:1 were tested. The extinction coefficients at 555 m μ were essentially

the same when the 1:9, 1:7, 1:5, and 1:3 ratios were employed. As the ratios were increased above 1:3, the absorption at 555 m μ decreased while the absorption at 338, 353, 373, 397, and 422 m μ increased. These maxima in and near the ultraviolet possibly indicate the formation of anhydrovitamin A.

Pyridine, aniline, *n*-butylamine, aqueous ammonia and epichlorohydrin inhibit the GDH-vitamin A color reaction. Aqueous KOH, ethanol, water and dioxane had some inhibitory action. Apparently compounds that react readily with HCl prevent the GDH-vitamin A color reaction.

Activated GDH reacted with β -carotene to form a product which absorbed strongly at and apparently above 1000 m μ . Above 880 m μ the stability of the extinction coefficients decreased with increasing wavelength at which measurements were made.

The GDH-vitamin A color reaction was readily quenched by adding ethanol. The ultraviolet absorption spectrum of the clear solution resulting when the reaction was quenched within 1 minute indicated the presence of a substance which absorbed at 335, 349, and 369 m μ . This absorption spectrum closely resembled that of isoanhydrovitamin A.

QUANTITATIVE SPECTROGRAPHIC ANALYSIS OF HAFNIUM-ZIRCONIUM MIXTURES¹

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In general, the analysis of mixtures of chemically similar elements by traditional analytical procedures is a difficult, if not impossible, task. The techniques of quantitative emission spectroscopy offer a particularly good solution to this problem, except that their use is usually restricted to the determination of small concentrations of elements. This thesis describes a spectrographic method of analyzing mixtures of two chemically similar elements, zirconium and hafnium, in concentrations from 0.1 to 99 per cent hafnium with an error of $\pm 1-2$ per cent. The successful analysis of higher concentrations of hafnium was shown to depend upon the use of (a) a nearly ideal internal standard, (b) line pairs which possessed similar excitation potentials, (c) lines which were susceptible to a minimum of self-absorption, (d) a controlled excitation source, and (e) certain refinements in the photographic photometry.

A Jarrell-Ash Wadsworth mounting 21-foot grating spectrograph was employed in this work. The external optical system was designed to focus the source on the collimating mirror. A rotating stepped sector was placed in front of the slit in order to achieve intensity gradations. An ARL-Dietert Multisource Unit was used as the exciting source with 14 microfarads, 480 microhenries, and 65 ohms in the power circuit. The spectra were photographed in the first order on Eastman Spectrum Analysis No. 1 emulsion and the emulsion response was determined by the two-step, preliminary curve method.

Preliminary studies showed that when an over-damped condenser discharge was used to excite $\frac{1}{4}$ -inch diameter pellets of zirconium-hafnium oxide and graphite, reproducible intensity ratios of hafnium lines to zirconium lines were observed. Moving plate studies showed that the ratio of intensities was constant with time. Since there was no selective volatilization of either element, it was possible to use one pellet for several determinations.

Standard samples were prepared from ZrO_2 containing some hafnium as an impurity and HfO_2 containing some zirconium as an impurity. The amount of impurity in each was determined by the method of zero intercepts. Pellets weighing 500 mg. were made of this material and graphite powder in the ratio of 1:4. At low hafnium concentrations an oxide-graphite ratio of 1:1 was used.

¹ Doctoral thesis number 1068, submitted June 5, 1950.

Seven line pairs were used for the concentration range. Except at very low and at extremely high hafnium concentrations, each was useful over a short concentration range, i.e., their intensity ratio was between 0.6 and 1.7 over this range. Such a procedure minimized the errors in photometry. Line pairs which were selected for quantitative calibrations possessed similar excitation potentials; were relatively free from interferences; and, with one exception, were within 25 Å from one another. Straight lines which had slopes near the theoretical slope of one resulted when log intensity ratio was plotted against log concentration ($\text{Hf/Zr} \times 100$).

Studies were made on the effect of changing the experimental variables. It was found that the graphite-oxide ratio, the crystal structure of the oxide, and the base material itself had little or no effect on the intensity ratios. The effect of additions of extraneous materials in the form of sodium chloride and calcium up to the amount of 5 per cent was investigated and was found to be negligible. It was found that line pairs which possessed unfavorable excitation characteristics likewise were unaffected by these latter additions.

A study was made of the effect of changes in excitation on line pairs possessing similar and dissimilar excitation potentials. Those line pairs which had nearly the same excitation potentials were relatively constant throughout wide changes in excitation, but those line pairs whose excitation potentials varied by a two-fold difference exhibited wide, erratic variations throughout the same range of excitation conditions.

The precision of the line pairs used in the quantitative calibrations was found to average 1.5 per cent standard deviation. A line pair possessing unfavorable excitation characteristics showed a per cent standard deviation of 5.05.

The accuracy of this method was determined by comparisons with independent spectrographic methods, with synthetic standards made up by an independent laboratory and with samples which had been analyzed by a chemical (atomic weight) method. The former two agreed within 1-2 per cent of the amount present. The latter determinations, made at an independent laboratory, averaged, with one exception, about 5 per cent higher than the spectrographic results. This discrepancy may be due to impurities in the selenium dioxide which was used in the chemical analysis.

FACTORS IN THE BIOSYNTHESIS OF TRYPTOPHAN AND TYROSINE BY *LACTOBACILLUS ARABINOSUS*¹

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PART I. STUDIES ON THE CONVERSION OF ANTHRANILIC ACID TO TRYPTOPHAN BY *LACTOBACILLUS ARABINOSUS*

Lactobacillus arabinosus is capable of converting anthranilic acid to tryptophan, but unable to synthesize the latter compound. It is therefore a promising organism for study of the later steps in tryptophan synthesis without interference from earlier reactions.

In synthetic medium lacking tryptophan, anthranilic acid is converted to tryptophan in good yield when added at low levels in the presence of pyridoxine or pyridoxamine. Higher levels of the acid inhibit growth. The anthranilic acid response curve is characterized by regular increase in growth to about 100 micrograms per 10 milliliter culture steady decrease to about 500 micrograms, nearly complete inhibition from 1000 to 5000 micrograms, and fair growth above 10,000 micrograms. The inhibition is prevented by low concentrations of indole or tryptophan or by allowing initiation of growth at lower levels of anthranilic acid. Although added at normally inhibitory levels, anthranilic acid is then converted to tryptophan and heavy growth ensues.

The inhibition is believed to involve interference with some essential cellular process, possibly a step in the conversion of anthranilic acid to tryptophan. Neither *p*-aminobenzoic acid nor nicotinic acid prevents inhibition by anthranilic acid.

A mutant strain overgrows inhibited cultures in 2 to 3 days. This mutant apparently differs from the parental strain only in its insensitivity to anthranilic acid at levels inhibitory to the latter strain.

Several compounds which are related structurally to anthranilic acid, indole, or tryptophan were tested for tryptophan activity and for ability to inhibit the conversion of anthranilic acid to tryptophan. A group of *N*-substituted anthranilic acid derivatives—ethyl, methyl, acetyl, formyl, and carboxymethyl—replaced tryptophan to varying degrees, apparently by conversion to anthranilic acid. On prolonged incubation, *N*-carboxymethylanthranilic acid appears as active as the parent acid itself. The activity of the formyl derivative (about 6 per cent) is enhanced by autoclaving. The other derivatives possess only slight activity. 5-Chloro- and 5-methylanthranilic acids showed no significant activity either in replacement of tryptophan or inhibition

¹ Doctoral thesis number 999, submitted August 22, 1949.

of its synthesis. Similar results were obtained with *o*-chloro-, *o*-bromo-, and *o*-methylbenzoic acids, skatole, indoleacetic acid, and cinnamic acid.

Attempts to obtain conversion of indole to tryptophan by nondividing cell suspensions were successful.

PART II. TWO MUTATIONS AND A STRUCTURAL ANALOG WHICH AFFECT SYNTHESIS AND METABOLISM OF ARYLALANINES IN *LACTOBACILLUS ARABINOSUS*

Two *L. arabinosus* mutants, one able to synthesize tyrosine and the other synthesizing both tyrosine and phenylalanine, were isolated. (Both mutations had been observed previously.) The relation of such mutations to the hypothesis that unused biochemical abilities are normally lost was discussed.

Quantitative comparison of the requirements of parental and mutant strains for phenylalanine and tryptophan indicated that neither step of the sequence tryptophan \rightarrow phenylalanine \rightarrow tyrosine, which has been reported for *Escherichia coli*, is utilized by the *L. arabinosus* mutants.

Inhibition of growth of *L. arabinosus* by *p*-fluorophenylalanine is reversed competitively by phenylalanine with a 50 per cent inhibition ratio (20 hours) of about 0.7. Tyrosine is without effect on the inhibition of parental or either mutant strain. This amino acid is therefore not a precursor in synthesis of phenylalanine by the mutant which performs this synthesis. The time course of the inhibition was followed, and the dependence of such values as inhibition ratios and antibacterial indices on time of incubation was discussed. In the presence of low levels of phenylalanine, small amounts of the fluoro derivative enhanced growth. Neither phenylalanine nor tyrosine is antagonized by *p*-chlorophenylalanine.

Increasing the molar ratio of fluorophenylalanine to phenylalanine causes a decrease in rate and extent of growth until a critical ratio (about 2) is reached. At levels of analog above this ratio, visible growth occurs only after a marked time lag, and appears to consist of a mutant strain resistant to the inhibitor. The lag period tends to vary with the level of inhibitor.

Increased specificity for phenylalanine of the enzyme mediating the block reaction is proposed as the most likely mechanism of the mutation. The implications of such a mechanism for the development of drug resistance and of enzyme specificity in general are discussed.

The three mutants studied, as well as one reported in Part I, are suppressed in some manner by the parental strain under conditions allowing only moderate growth of the latter strain. This suppression is denoted by the occurrence of a depression in total growth under conditions intermediate between those allowing normal growth of the parental strain and those causing clean selection of the mutant. The mechanism of this suppression is not known.

SOME FACTORS AFFECTING QUANTITY AND QUALITY OF EGGS MARKETED BY CERTAIN PRODUCERS ¹

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The objectives of this study were: (1) to determine how the egg marketing operations of selected egg producers and handlers in Iowa compare to certain portions of what may be considered a perfect market; (2) to determine the effect of cost-price relationships on the quantity of eggs produced in the United States; (3) to determine the relationships of the egg production and marketing on record-keeping farms in Iowa to the farm as a production unit and to the household; and (4) to determine what record-keeping farmers in Iowa consider to be the most important factors affecting quantity adjustments in egg production.

The results of this study indicated that egg producers in Iowa were generally confronted with a marketing system which did not reflect market demands from a form (grade) standpoint. There was an indication that place price differentials existed. Sales during the fourth quarter of the year were an important price-factor among record cooperators. There was also slight indication of price differentials for what might be called quantity utility.

The form (grade) imperfection in the market for Iowa egg producers was quite apparent. There was a general tendency for handlers to buy eggs on a smaller number of grades than the number on which they sold the eggs.

The major effect on Iowa producers of grade buying was the lowering of the percentage of dirty eggs sold by producers. The interior quality of eggs was considerably higher during the summer months and somewhat higher during the spring months for eggs sold on a graded basis than those sold on an ungraded basis. The use of farm routes did not result in higher quality of eggs as compared to door deliveries. In some instances, door-delivered eggs were of higher quality than farm-route eggs. No comparisons were made between dealer-route eggs and farm-route eggs. There was no apparent relationship between the quantity and quality of eggs sold by producers.

There was little apparent quality deterioration from the time the eggs were graded at the first-buyer's plant until they were graded at the carlot shipper's plant. This low deterioration may have been due to the relatively short time interval between gradings.

¹ Doctoral thesis number 998, presented August 22, 1949.

A sample of 1100 Iowa producers indicated that those who sold eggs on a graded basis received a significantly higher price per dozen eggs in August 1948 than producers who sold on a current receipts basis. Farm record cooperators in Iowa who sold eggs on a graded basis in 1947 also received higher prices than those who sold eggs on a current receipts basis. In the latter group, method of sale appeared to be of greater importance in price determination than the quality maintenance practices followed.

Egg producers in the United States responded to changes in cost-price relationships by raising more or fewer flock replacements. The indicator of cost-price relationships was the ratio of the value of the eggs produced by the average hen to the cost of 1 pound of poultry ration. More than 93 per cent of the variance in the number of potential layers on U. S. farms on October 1, 1930 to 1947 was associated with changes in the number of potential layers on farms the preceding October 1 and changes in a weighted value of eggs produced per hen-feed price ratio. Nearly 86 per cent of the variation in the number of pullets on U. S. farms on October 1, 1930 to 1947 was associated with changes in the above ratio. Although the value of eggs produced per hen-feed price ratio was a good indicator of the initial size of laying flock, only about 25 per cent of the rate of decline in the number of hens on U. S. farms was associated with this cost-price measure. Nearly 99 per cent of the variance in October 1 to September 30 egg production for the period 1930-31 to 1946-47 was associated with changes in the number of potential layers on farms and egg production per hen in October.

Increased egg production per hen was an important factor in increased total egg production. The price of eggs relative to hog prices decreased from 1925 to 1948 but the ratio of the number of eggs to the number of pigs saved increased. There was no indication, from an aggregate standpoint, of competition between hog production and egg production in either Iowa or the United States.

Among a sample group of farm record cooperators in Iowa, egg production was higher on the average size farms than on either the smallest or largest farms. Owner-operators kept more hens than did tenant-operators. This was a partial reflection of the better housing facilities provided on owner-farms. Operators in the 41-50 age group kept more hens than either younger or older operators. Hog and egg production were considered competitive by a few operators. There was apparent competition between specialized dairy or beef production and egg production.

The exact contribution made toward maximization of managerial return by family care of the poultry flock was not apparent. The number of children of ages 13 to 18 was closely associated with the number of hens kept on these farms, however. There was a greater percentage of flocks in the 200 to 299 hen group among farms on which family labor had major responsibility for the care of both hens and chicks.

Among the farm record cooperators in Iowa, producers selling eggs on a graded basis had larger flocks and received a greater percentage of their cash income from the sale of eggs than those selling on an ungraded basis. There was little relationship between other portions of the farm enterprise and marketing practices followed.

The record-keeping farmers in Iowa considered housing facilities to be the most important factor affecting the number of chickens to raise. This is a short-run consideration since housing facilities could be provided on owner farms if the long-run outlook for egg prices appeared to be particularly favorable. Cost-price factors were ranked as second in importance and competitive factors were seldom included.

Many of these producers changed breeds of hens during the last five years in an attempt to get greater egg production. Many of them also bought sexed pullets because of the relatively greater returns from eggs than from chickens. There was little indication that cost-price relationships, as such, were of any importance in determining when to sell hens.

Among a group of ninety-eight farm record cooperators in Iowa with 10-year records, producers who decreased the hen flock size by 100 to 199 birds from 1937 and 1938 to 1946 and 1947 increased their management return significantly more than producers who increased their laying flock size by 100 or more birds. However, the size of the laying flock, per se, was not an important factor in determining management return.

OXIDATION OF FATTY ACIDS BY *CANDIDA LIPOLYTICA* AND *PSEUDOMONAS FRAGI*¹

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The ability of microorganisms to break down fats and utilize the resulting products in their metabolism has been recognized for some time; however, microbial lipases have been studied in greater detail than have the microbial oxidases. The present investigation was undertaken to study the ability of *Candida lipolytica* and *Pseudomonas fragi* to oxidize sodium salts of fatty acids, and various nutritional and environmental factors which may affect this oxidation.

Highly oxidative strains of *C. lipolytica* and *Ps. fragi* were selected by using butterfat-emulsion agar plates and flooding the plates with an aqueous solution of tetramethylparaphenylenediamine hydrochloride, after 4 days growth at 21°C. The lipolytic ability of these strains was determined by growth for 4 days at 21°C. on butterfat-emulsion agar plates to which Nile blue sulphate had been added. The *Ps. fragi* strain selected was atypical since it exhibited no lipolytic activity.

The cells were propagated on tryptone-glucose-extract agar slants, harvested by washing from the agar with chilled saline and the centrifugation and washing repeated three times. The cell suspensions were adjusted to a constant reading of 250 with chilled saline, using a Klett-Summerson photoelectric colorimeter.

The ability of resting cell suspensions of these microorganisms to oxidize the sodium salts of acetic, propionic, butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, oleic, and linoleic acids at various pH levels was determined using conventional Warburg respirometer technics. Pure fatty acids were preferred to natural fats or monoglycerides as substrata since they had no glycerol component which could be oxidized to yield misleading results. Furthermore if fats or monoglycerides were used, the lipolytic ability of the organisms tested would be the limiting factor, in some instances, in making the fatty acids available for oxidation. Selected fatty acids were used as test substrata in other phases of this study.

The buffers selected for use in the reaction flasks of the Warburg apparatus were chosen on the basis that they would cover a wide pH range, that they were not highly toxic to the microorganisms used and that they would maintain the pH relatively constant at each pH level tested for a 2.5-hour run. A 0.05M McIlvaine citrate-phosphate

¹ Doctoral thesis number 1031, submitted March 8, 1950.

buffer was selected for *C. lipolytica* and a 0.07M Palitzsch borate buffer for *Ps. fragi* cells.

Incubation of *C. lipolytica* and *Ps. fragi* cells for 24 hours at 30°C. and 21°C., respectively, gave the highest oxygen uptake values, and were used in this investigation. A run covering the period from 0.5 to 2.5 hours gave higher uptake values than a run of 0 to 2.0 hours; accordingly the 0.5- to 2.5-hour interval was used in this investigation.

The action of *C. lipolytica* cells upon the twelve test substrata previously listed indicated the optimum range of reaction to be pH 3.4 to pH 7.8, with the optima for the majority of the substrata falling within the range of pH 5.8 to 6.6. This pH range agrees closely with the pH range found to be optimum for the lipase enzyme of *C. lipolytica*.

The action of *Ps. fragi* cells upon the twelve test substrata indicated the range of greatest activity to be pH 8.0 to pH 8.45, with the majority of the substrata being oxidized most readily within the range of pH 8.25 to 8.45. This pH range is in close agreement with the optimum pH values found for various bacterial lipases.

Cells of *C. lipolytica* grown on tryptone-glucose-extract agar adjusted to pH 3.8 to 4.4 showed higher oxygen uptake values than cells grown on agar adjusted to higher pH levels. The cells of *Ps. fragi* grown on tryptone-glucose-extract agar adjusted to pH 5.0 to 7.8 showed higher uptake values than cells grown on agar adjusted to lower pH levels.

In the present study there is some evidence of an enhancement of oxidase production by *C. lipolytica* and *Ps. fragi* when grown in the presence of certain added nutrients. This is not a true adaptive enzyme production in the usual sense, because the cells grown in the absence of the fatty acids possessed oxidizing ability to a considerable degree.

Suitable synthetic media were chosen for the cultures used, when studying the effect of added nutrients upon oxidase production. The presence of such added complex nutrients as skimmilk, peptone, casein hydrolysate, yeast extract or beef extract caused no appreciable enhancement of oxidase production by either *C. lipolytica* or *Ps. fragi*. Cells of *C. lipolytica* grown in the presence of 20 per cent cream gave higher uptake values than the cells grown in the presence of skimmilk, indicating an enhancement of oxidase production in the presence of butterfat. The failure of cream to enhance the production of oxidase enzymes by *Ps. fragi* undoubtedly is due to the inability of the test culture to hydrolyze butterfat to a detectable degree. In the absence of free fatty acids the stimulus for increased production of enzymes oxidizing these acids would be lacking.

The addition to the growth media of fatty acids such as acetic, propionic, butyric, or caproic resulted in definite enhancement in the ability of both *C. lipolytica* and *Ps. fragi* to produce oxidase enzymes. Some tendency toward specificity of the enzymes responsible for oxygen uptake seems apparent since the stimulation for utilization of one acid sometimes was not apparent to the same degree for all other acids in

the homologous series. Acetic acid appeared to behave quite differently from butyric acid in that the latter stimulated to a much greater degree the production of oxidases active against the fatty acids which usually are found as components of natural fats; propionic acid also had some tendency to be less active than butyric acid in stimulating production of fatty acid oxidases.

The addition of monoacetin and monobutyrim to the growth media appeared to have little effect in enhancing the oxidizing ability of either *C. lipolytica* or *Ps. fragi* when tested against the sodium salt of the fatty acid present in the monoglyceride. Cells grown in the presence of monobutyrim showed uptake values appreciably higher than the control values when myristate was the test substrate. When caproate was used as the test substrate for cells grown in the presence of either of these addenda, no detectable increase over the control values was noted.

An enhancement of the oxidizing ability of cells of both *C. lipolytica* and *Ps. fragi* when grown in the presence of fatty acids and natural fat gives proof that these cells react to the stimulus of the presence of a fatty acid by increasing the amount of enzyme available for the utilization of the substrate. Natural fat added to the growth medium gave no increase with *Ps. fragi* cells, probably because the strain of *Ps. fragi* chosen for this study was non-lipolytic.

In dairy products containing butterfat both *C. lipolytica* and the normally-lipolytic forms of *Ps. fragi* would be stimulated to produce considerable quantities of oxidases active on fatty acids, whether these fatty acids resulted from the lipolytic activity of the organisms, synthesis by other microorganisms or by the action of milk lipases.

GROWTH OF THE MAIZE ROOT TIP ¹

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Growth of the primary maize root has been found to be limited to the first 4 millimeters behind the root cap. The division zone, defined as that fraction in which anticlinal divisions are taking place, has been further separated into two rather distinct portions. One of these is formed by the group of cells located in the first millimeter above the root cap base, and is characterized by slow growth, small-sized and non-vacuolated cells. This section is rounded and somewhat tapered, so that it was in our tests, 40 per cent smaller than the second millimeter section. The cells of the first section were found to be isodiametric, 11 μ long, high in protein (61 per cent of the dry weight) and dry matter (about 20 per cent). Generation time for one complete cell division was 29 hours at 15° and 18 hours at 25°C.

In the second millimeter a rapid change from cell division to cell enlargement occurred. The first quarter of this section was similar to the preceding region except that cell division was probably accelerated by higher temperature. The maximum rate of cell division, with a generation time of less than 10 hours and possibly as low as 6 hours was estimated for the second quarter of this region. At the same time, the average cell length nearly doubled to give a sharp peak of total growth. Enlargement continued and cell division slowed in the third and ceased in the fourth quarter of the second millimeter. Average protein nitrogen per cell in the second millimeter was double that in the first and average cell length was not quite double. Rapid protoplasm synthesis was indicated.

The third millimeter grew by enlargement only, at a rate one-third less than the combined effects of cell division and enlargement in the second section. Total nitrogen per cell unit doubled again over the second section but cell length increased merely three times. Protein synthesis began to lag. The hydration index (water/dry matter) increased from 4.2 in the first millimeter to 5.2 in the second and 6.8 in the third. This increased hydration was accompanied by an accumulation of soluble nitrogen and sugars which would tend to induce hydration by vacuolation. The change in the hydration ratio reached a maximum between the third and fourth millimeters where it jumped from 6.8 to 11.8. At the same time, dry matter, protein, ash, and most cell

¹ Doctoral thesis number 1042, submitted March 14, 1950.

constituents except sugar and cellulosic materials decreased on a cell unit basis, indicating rapid vacuolation.

Because of the inevitable time lag in growth measurements, the enlargement rates assigned to the third millimeter occurred for the most part in the fourth millimeter. For the same reason the low growth totals shown for the fourth millimeter mean a short period before the fourth millimeter became the fifth, rather than a slow rate. Cell length increase between the fourth and fifth millimeter was the second largest observed. Since no measurable growth was recorded in the fifth millimeter, the changes in this and succeeding sections were considered to be confined to differentiation reactions, among which the increase of cellulosic materials was notable.

It has already been indicated (1) that the rates of cell division in the first and second millimeters were increased by temperature changes from 15 to 25°C. Specific division rates for the two temperatures were 0.035 and 0.055 in the first millimeter, and 0.036 and 0.092 in the second, over a 3-hour interval. A possible rate of 0.126 was estimated for the distal part of the second millimeter.

The rate of cell enlargement also showed high temperature coefficients; on the order of 3-4 for a rise of 10°C. in the third millimeter where no cell division occurred. This coefficient indicated that chemical rather than physical reactions were limiting for cell elongation processes (2). Root tip sections held in aerated water showed small water absorption. The same sections held in 2 per cent sucrose solution absorbed a little more water than the checks, but sections in indoleacetic acid, and particularly in indoleacetic acid plus sucrose increased 50 per cent in fresh weight in one hour and their hydration ratio doubled. Cell enlargement would seem to be limited by enzymatic reactions affected by the presence of auxins. These reactions possibly involved the lengthening of the cellulose micelles of the cell wall in such a way as to allow further expansion of the entire cell.

Division of rapidly vacuolating cells was not detected in the meristematic tissues of primary maize roots. This observation suggests the occurrence, at specific radicle levels, of irreversible changes in the nature of the meristematic development. The protein material in the apical two millimeter section was relatively dense, as shown by the small cells with their high protein content and viscosity. With hydration, the structure of the proteins may be irreversibly altered, somewhat in the manner shown *in vitro* (4) for films of a number of proteins. The general level of metabolic and particularly of structural activity might be reduced by the tendency of protein molecules to unfold when hydrated. Such changes in protein structure may account for the failure of hydrated protoplasm to continue division.

AVAILABLE COMPOUNDS

Total reducing substances after hydrolysis with invertase showed the expected gradient, being highest nearer the endosperm source and

lowest in the apical section. Soluble nitrogen, which may be considered to be a translocation form, did not show the same pattern but was highest in the third millimeter section and lower both apically and basally from this region. Concentrations here suggested synthesis of proteins in the zones of division and digestion in the third and later zones. There is evidence (3) that storage proteins are retranslocated only after digestion to simpler water soluble forms and that the various soluble organic materials are more or less interchangeable in their effects on growth. A sharp decline in soluble nitrogen with the corresponding increase in colloidal nitrogen may be indicative of optimal environmental conditions required for the condensation of simple nitrogenous compounds into proteins and new protoplasts.

RESPIRATION

Respiration measurements on the isolated meristematic regions of primary maize roots led to the differentiation of each growing region in terms of their distinct respiratory activities. Endogenous respiration tended to be highest in the zone of cell enlargement, but differences were surprisingly small and the rate in the zone of differentiation was almost as high as either of the others. Exogenous respiration rates showed that the relatively high rate of the differentiation zone was due to its high sugar content. The endogenous respiration decreased consistently with time after cutting. This trend suggested that the endogenous rate of respiration depended largely on a steady supply of substrate from the seed. The greater susceptibility to cyanide and iodoacetate inhibition in the enlargement zone indicated basic differences in the nature and activity of the respiratory systems operating in these zones.

SUMMARY

The temperature coefficients for cell enlargement, the relatively large accumulation of soluble nitrogen, the high respiration rate and the sensitivity to inhibitors, provided several indications that the meristematic regions of primary maize roots undergoing cell enlargement included the most active centers of the root in many metabolic functions.

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NEW TITRIMETRIC METHODS FOR THORIUM¹

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Of the large number of methods which have appeared in the literature for the determination of thorium, practically all are based on precipitating the thorium in some form from an acid or neutral solution and completing the determination by a gravimetric method. The precipitate is either ignited to the dioxide directly or dissolved and the thorium precipitated as the oxalate after which it is converted to the dioxide by ignition. The work being reported, in which successful titrimetric methods for thorium are described, was prompted by the need for a rapid and accurate method for determining thorium in various alloys and mixtures.

ADSORPTION INDICATOR METHODS

Thorium nitrate solutions were titrated with various precipitating agents such as ammonium paramolybdate, phenylarsonic acid, ammonium phosphate, and potassium pyrophosphate using such indicators as methyl violet, phenosafranin, rhodamine 6G, and tartrazine. None of the above combinations gave a satisfactory end-point.

OXIDIMETRIC MOLYBDATE METHOD

A new titrimetric method for thorium has been developed which is based on the quantitative precipitation of thorium as the normal molybdate and the subsequent reduction and titration of the molybdenum that is combined with the thorium.

The general procedure for the determination of thorium is given below. Samples containing 0.15 to 0.2 g. thorium dioxide are weighed out and placed in 250 ml. beakers. After the samples have been dissolved any large excess of mineral acid is destroyed by evaporating the solutions nearly to dryness. The samples are then diluted to 150 ml. with water and made about 7 per cent in acetic acid by adding 11 ml. of glacial acetic acid. Fifteen ml. of thick filter pulp and 1 ml. of a diphenylcarbazine solution (0.5 g. per 200 ml. 95 per cent ethanol) are added. The ammonium paramolybdate solution (7.6 g. per liter) is added from a buret with stirring until the indicator imparts a deep pink color to the solution. After the precipitates have settled the supernatant liquid may be tested for complete precipitation. The contents of the beakers are heated to boiling and filtered while hot through

¹ Doctoral thesis number 811, submitted August 21, 1946.

11 cm. Whatman No. 42 filters into 400 ml. beakers. The precipitates are washed 5-6 times with hot 1:100 acetic acid. The 250 ml. beakers need not be scrubbed out with a policeman but only carefully rinsed out 2-3 times with wash solution. The washed precipitates and filters are transferred to the 250 ml. beakers in which the precipitations were carried out and 25 ml. of concentrated hydrochloric acid added to each beaker. The contents are stirred until the filters disintegrate. Seventy-five ml. of water is added, the mixtures heated to boiling (long boiling results in reduction of molybdenum and decomposition of the filter pulp) and filtered, while hot, through 11 cm. Whatman No. 42 filters into 400 ml. beakers. The filter pulp and filters are washed 5-6 times with hot 1:100 hydrochloric acid. The filtrates after being cooled to room temperature, are passed through an amalgamated zinc Jones reductor into an excess (five times the theoretical of 10 per cent) of ferric alum to which 2-3 ml. of concentrated phosphoric acid has been added and titrated with 0.1 N ceric sulfate using two drops of ferroin as indicator. The end-point is taken as that point when the pink color of the solution changes to colorless or blue.

This method has been found to be very useful in the separation of thorium from calcium and uranium. One hundred seventy mg. of thorium dioxide can be separated from as much as 400 mg. of calcium and determined to within less than two parts per thousand. The addition of ammonium acetate is necessary in the case of uranium to prevent the precipitation of uranyl molybdate. The same amount of thorium dioxide can be separated from 200 mg. of uranium oxide, U_3O_8 , and determined to within four parts per thousand.

The separation of thorium from rare earths such as lanthanum, cerium, samarium, neodymium, and also yttrium was tried with various acetic acid concentrations and at various temperatures but quantitative separation was not obtained.

Molybdenum can be determined by reversal of the above method. Thus 140 mg. of molybdenum trioxide, MoO_3 , can be conveniently separated from 240 mg. of uranium oxide, U_3O_8 , by precipitating it as thorium molybdate from a 7 per cent acetic acid solution containing ammonium acetate. The molybdenum can be determined to within three parts per thousand.

ELECTROMETRIC METHODS

The possibility of detecting the end-point electrometrically when thorium is titrated with ammonium paramolybdate was investigated and found to be very promising. Of the various conditions tried for this titration the best was a 7 per cent acetic acid solution at 50°-55° with a 0.1 N calomel reference electrode and a molybdenum wire indicator electrode.

This electrometric method was found to be very useful for determining thorium in the presence of calcium. One hundred fifty mg. of thorium dioxide can be determined in the presence of as much as 400 mg. of calcium with an error of about three parts per thousand.

OXIDATION OF L-TYROSINE BY LIVER TISSUE¹

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Previous work on the metabolism of L-tyrosine has failed to disclose many of the important features involved. In the present work the fate of the nitrogen of tyrosine when the amino acid is oxidized by normal guinea pig liver tissue is the primary issue. As a tool for studying this problem, tyrosine containing isotopic nitrogen (N^{15}) has been synthesized.

The method of synthesis was an adaptation of the "azlactone synthesis" of Herbst and Shemin, by which they synthesized phenylpyruvic acid. Since the intermediate required for the introduction of isotopic nitrogen for our work was *p*-hydroxyphenylpyruvic acid, the following modifications were made: 0.37 moles of *p*-hydroxybenzaldehyde, 0.4 moles of acetyl glycine, 0.3 moles of anhydrous sodium acetate and 1.33 moles of acetic anhydride were refluxed for five hours to produce the corresponding azlactone, 2-methyl-4- (4'-acetoxybenzal)-5-oxazolone. This compound was converted to *p*-acetoxy- α -acetamino-cinnamic acid by refluxing with acetone and water. Hydrolysis of this with 1 *N* hydrochloric acid afforded the desired *p*-hydroxyphenylpyruvic acid. The yields of these three steps were 66, 94, and 53 per cent, respectively.

The isotopic nitrogen was introduced into the molecule by catalytic reduction of the *p*-hydroxyphenylpyruvic acid at low pressure in the presence of ammonia containing N^{15} . This produced N^{15} -DL-tyrosine in 73-90 per cent yields.

Since the natural L-isomer was desired for our work, an attempt was made to resolve the isotopic DL-tyrosine by converting it to acetyl-DL-tyrosine and fractionally crystallizing the brucine salt. This method was not satisfactory under the conditions employed, and a new resolution method was developed. This new method involved fractional crystallization of the salt N^{15} -acetyl-L-tyrosine-*d*- α -phenylethylamine from absolute ethanol. This salt was obtained in 43-60 per cent yields in optically-pure form after only two or three crystallizations. From this salt the N^{15} -L-tyrosine was obtained in 76 per cent yield.

The N^{15} -L-tyrosine was incubated with normal guinea pig liver homogenate in the Warburg respirometer. The deproteinized incubation mixture was then fractionated and the different fractions investigated for isotopic nitrogen content. In an effort to prove or disprove a previous theory that the amino acid, alanine, is formed on an equimolar basis when tyrosine is oxidized by liver tissue, normal alanine was added to

¹ Doctoral thesis number 1006, submitted November 23, 1949.

the incubation mixture as a carrier and alanine then isolated as the salt of azobenzene-*p*-sulfonic acid. This derivative was purified and the N^{15} content determined on the mass spectrometer. Other fractions analyzed included a room-temperature precipitate with the azobenzene-*p*-sulfonic acid, a fraction adsorbed to barium acetate and barium sulfate, and butanol-soluble and butanol-insoluble fractions.

The atoms per cent excess N^{15} of each fraction was converted to milligrams of excess N^{15} in order to calculate the yield of isotope found in each fraction. All fractions had negligible isotope content except the butanol-insoluble fraction, which had 20.99 per cent of the isotope present in the reaction, and the alanine-azo-benzene-*p*-sulfonate salt, which had 99.84 per cent of the isotope present. The butanol-insoluble fraction probably included some unreacted N^{15} -L-tyrosine.

Previous experiments with tyrosine labeled with radioactive carbon in the α -position of the side chain had proved conclusively that the side chain of tyrosine and part of the benzene ring contribute to acetoacetic acid formation in the oxidation of this amino acid. Therefore there are only two possibilities for formation of alanine from tyrosine under normal oxidative conditions. One, oxidative deamination to form *p*-hydroxyphenylpyruvic acid and ammonia; and two, transamination, in which the amino group is passed from the tyrosine to α -ketoglutaric acid to form glutamic acid, and from the glutamic acid to pyruvic acid to form alanine. In the first case, the ammonia so formed presumably would react with pyruvic acid to form the alanine. The presence of glycogen in liver homogenates has been noted in our experiments. The large uptake of oxygen upon incubation of liver homogenates without added amino acid as substrate indicates a very active glycolysis, which would provide more than adequate concentrations of α -ketoglutaric acid and pyruvic acid for either mechanism suggested.

Feeding experiments also were conducted with the N^{15} -L-tyrosine. A guinea pig was fed a scorbutigenic diet supplemented with the isotopic tyrosine, a scorbutigenic diet supplemented with isotopic tyrosine and ascorbic acid, and the same diet supplemented with isotopic tyrosine and pteroylglutamic acid. The urine was fractionated into ammonia, urea, and residual nitrogen for isotope analysis.

In all three cases insufficient ammonia was excreted to analyze the N^{15} content. In the control and the vitamin C-supplemented experiments, the isotopic contents of the urine were essentially the same, as were the two urea fractions. The urine from the pteroylglutamic acid-supplemented guinea pig contained about 12 per cent more isotope than did the control; most of this increase was in the urea fraction. It was evident from these preliminary experiments that although vitamin C exerts an influence on the complete oxidation of tyrosine, it apparently does not operate in the step which detaches the nitrogen from the amino acid. Then it is apparent that the mechanism of action of the two vitamins in the tyrosine oxidation scheme is different.

SUMMARY

1. The synthesis of DL-tyrosine containing isotopic nitrogen has been effected in good yields by a four-step process: the azlactone synthesis (52-66 per cent yield); breaking the ring by mild hydrolysis with acetone and water (71-94 per cent yield); acid hydrolysis to obtain *p*-hydroxyphenylpyruvic acid (48-53 per cent yield); and catalytic hydrogenation of the substituted pyruvic acid in the presence of isotopic ammonia (73-90 per cent yield).

2. N¹⁵-Acetyl-DL-tyrosine has been resolved by the use of *d*- α -phenylethylamine to give yields of 43-60 per cent of the salt of the L-isomer, from which N¹⁵-L-tyrosine was isolated in 77 per cent yield.

3. The isotopic tyrosine was oxidized *in vitro* by guinea pig liver homogenate, and alanine subsequently isolated from the reaction mixture as the azobenzene-*p*-sulfonate salt.

4. Isotopic analysis of the isolated compound showed that almost all of the N¹⁵ from the tyrosine was contained in the alanine derivative.

5. Isotopic tyrosine was fed to guinea pigs on scorbutigenic diets with and without supplements of ascorbic acid and pteroylglutamic acid, and the urine and feces examined for isotopic nitrogen content. These experiments indicated that the vitamins apparently have little effect on the disposal or fate of the amino-nitrogen of tyrosine.

EFFECT OF BENZENE AND SIX SELECTED SALICYLATES
ON THE DEVELOPMENT OF IMMUNITY IN
TRYPANOSOMA LEWISI INFECTIONS¹

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A study was made of the effect of benzene, ethyl alcohol², and six salicylates on the development of immunity to *Trypanosoma lewisi* infection in albino rats. Certain aspects of the blood picture were followed.

Bartonella-free, Wistar A rats were infected with Becker's strain of *Trypanosoma lewisi* by the intraperitoneal injection of diluted blood containing over 100,000 parasites. Methyl salicylate (0.1 to 0.2 ml.), acetylsalicylic acid (45 to 98 mg.), para-aminosalicylic acid (50 to 100 mg.), phenyl salicylate (45 mg. per 100 g.), sodium salicylate (45 mg. per 100 g.), salicylic acid (45 mg. per 100 g.), benzene (approximately 0.2 ml. per 100 g.), benzene (0.1 to 0.2 ml. per 100 g.) plus sodium salicylate (45 mg. per 100 g.), or 35 per cent ethyl alcohol (2.0 ml.) was administered daily throughout the course of the infection to test rats by stomach tube. Test substances were suspended in a 12 per cent aqueous solution of gum arabic or were dissolved in distilled water or 35 per cent ethyl alcohol. Comparable amounts of the carrier substance were administered daily to *Trypanosoma lewisi*-infected control rats by stomach tube.

Tail blood was examined on the day of inoculation of trypanosomes, on the third day of the infection, and on alternate days thereafter. Total trypanosome counts were made by the standard hemacytometer method for counting leucocytes. Hemoglobin concentration of the blood was determined with the use of a Spencer Hb-meter. Blood smears were stained with Wright's blood stain and were used in making differential leucocyte counts, determinations of the percentage of dividing trypanosomes (blepharoplast dividing or divided), and, occasionally, calculations of standard deviation for total length of trypanosomes. Standard deviation was calculated by the method of Taliaferro and Taliaferro (1922) (1). Total trypanosome counts served as an index of the effectiveness of the trypanocidal antibody. The percentage of dividing parasites (division forms) and the standard deviation for total length

¹ Doctoral thesis number 1035, submitted March 11, 1950.

² Ethyl alcohol was administered to a few test rats, since it was used as the solvent for acetylsalicylic acid in two experiments, and there was some question as to the effect it might have on the development of immunity.

of trypanosomes served as indices of the anti-reproduction (parasite) resistance factor, ablastin.

Control rats responded to the protozoon by developing three types of immune bodies: (1) Taliaferro's ablastin, which appeared early in the infection and prevented further multiplication of the trypanosome; (2) Taliaferro's trypanocidal substance, which appeared within a short time after ablastin had produced its effect, and killed many of the adult trypanosomes by lytic action; and (3) a trypanosome-agglutinating substance, which became effective about the same time as the trypanocidal factor and also seemed to be instrumental in removing the adult trypanosomes from the blood stream.

Infections in para-aminosalicylic acid-treated rats and in ethyl alcohol-treated rats were essentially like control infections. All other test substances altered the typical course of the trypanosome infection by affecting the development of host-resistance to the parasite.

Methyl salicylate, acetylsalicylic acid, phenyl salicylate, sodium salicylate, salicylic acid, and benzene plus sodium salicylate partially or completely inhibited both ablastic action and the agglutination of the trypanosomes in the blood. The above test substances seemed to have no effect on the trypanocidal factor. Infections in rats treated with the test substances listed above were characterized by continued reproduction of the parasites throughout the course of the infection and by extremely dense trypanosome populations, with the time of the peak of the infection delayed. The infection in many of the treated rats took a pathogenic course, either of the continuous or of the relapsing type. The infection was prolonged in test rats which recovered.

Benzene, alone, partially inhibited the production of the trypanocidal resistance factor but had no effect on the elaboration or action of ablastin or of the trypanosome-agglutinating substance. Benzene-treated infections were characterized by cessation of parasite reproduction at the expected time and by larger-than-control trypanosome populations. Benzene-treated infections were also definitely prolonged.

Certain deviations from the normal blood picture were noted regularly for the majority of *Trypanosoma lewisi*-infected rats, control as well as test, except for those treated with benzene or with benzene plus sodium salicylate. The deviations seemed to be attributable directly to the trypanosome infection rather than to the treatment except as the latter influenced the size of the trypanosome population. They included: (1) an occasional slight increase in hemoglobin; (2) a reduction in the hemoglobin concentration of the blood which seemed to be directly proportional to the density of the trypanosome population (also observed for rats treated with benzene plus sodium salicylate); (3) an initial leucopenia; (4) a leucocytosis which was evident by the peak of the infection and which persisted until the parasites disappeared from the blood; (5) a slight increase in the incidence of mononuclear leucocytes as the infection progressed; (6) a comparable slight relative decrease in neutrophils; and (7) a slight increase in the incidence of

basophils (also observed for rats treated with benzene or with benzene plus sodium salicylate). The incidence of eosinophils was unaffected by the trypanosome infection.

Benzene, alone, markedly affected the hemoglobin concentration of the blood and the total and differential leucocyte counts. Benzene treatment was accompanied by fluctuating increases and decreases in hemoglobin and by sharp reductions in the total leucocyte count and in the percentages of mononuclear leucocytes and eosinophils. The relative number of neutrophils increased as the percentage of mononuclears decreased. The incidence of basophils was unaffected by benzene treatment, but was affected by the trypanosome infection.

Benzene plus sodium salicylate indirectly affected the hemoglobin concentration of the blood by virtue of the sodium salicylate effect on immunity, which favored a large trypanosome population. The effect of benzene on hemoglobin was not evident. However, the typical benzene effects on total and differential leucocytes, described above, were noted, the incidence of basophils again being affected by the infection rather than by treatment with benzene plus sodium salicylate.

The literature review includes: (1) a discussion of the typical *Trypanosoma lewisi* infection in non-treated rats following intraperitoneal injection of the parasites; (2) the most important theories regarding the nature of the rat's immunity to the trypanosome; (3) general effects of salicylates and of benzene on mammals; and (4) the effect of salicylates, benzene, and alcohol on the development of immunity.

The work was supported in part by a grant from the Industrial Science Research Institute, Iowa State College.

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APPLICATION OF SPRAY DRYING PRINCIPLES TO THE DESOLVENTIZATION OF MISCELLA ¹

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The desolventization of miscella (mixtures of soybean oil and trichloroethylene) by application of the principles of spray drying was investigated to ascertain if this method could insure stripped oil of higher quality at an operating cost less than that of conventional methods. High quality oil depends, among other things, on a low fraction of red color which in turn demands that even rather short exposure to high temperatures be avoided. Vaporization from a particulate system occurs at the boiling point of the solvent under the conditions of atomization, and for the miscella studied the trichloroethylene solvent will evaporate at 52.5°C. under 20 inches Hg vacuum. If a correct particle path has been selected the remaining oil after vaporization of the solvent need not be heated appreciably above 55°C.

While spray drying has been applied to almost every conceivable case involving desiccating, concentrating, or desolventizing a solution or suspension, until the time of this investigation no known application had been made to the separation of vegetable oil from the solvent which had been used to extract it from the seed. The feasibility of such application was shown and the investigation should pioneer further work in this field. Consequently, an exhaustive literature survey was made to gather related material on the theory and practice of spray drying as an aid to future workers. Very few references on the transfer of heat to particulate systems were found which possessed sufficient merit to be included in the survey.

Of the several types of spray nozzles studied only one was found suitable for semi-pilot plant research. The essential feature of the hydraulic nozzle selected was its nonclogging performance at relatively low feed rates. It was concluded that a centrifugal disc atomizer would yield more satisfactory and uniform operation than any of the various other atomization devices.

The minimum dimensions of an operable spray chamber were found to be those of a cylinder 2 feet in diameter and 4 feet high, containing a cone bottom whose vertical axis measured 1 foot. For the small scale of operations employed it was found desirable to add an internal heating coil of copper tubing pressed against the inner surface of the upper portion of the chamber.

¹ Doctoral thesis number 1011, submitted December 10, 1949.

Direct superheated steam at temperatures to 357°C. was employed as the dessicant with no noticeable effect on the oil quality. Even though the optimum degree of contact between the steam and particle cloud was not attained, nevertheless the steam requirements were found to be about one-half those in conventional desolventization methods. Further investigations of the method by which the dessicant may be introduced to the particles should be made.

The relation between vapor pressure and temperature was studied for several concentrations of miscella between 30° and 90°C. Between concentrations of 0 and 20 weight per cent oil the vapor pressure above the miscella was found to be consistently higher than that for pure trichloroethylene alone. Above 20 per cent oil the vapor pressure decreased but still remained above that for pure trichloroethylene over a concentration range dependent on the temperature. Similar variation was noted in the relation of the refractive index to the concentration.

A section is included which brings together many properties and relations of pure trichloroethylene, pure soybean oil, and the system: trichloroethylene-soybean oil. Such grouping has not been done heretofore and will facilitate future work in the field.

PASTURAGE AND ENVIRONMENTAL TEMPERATURE EFFECTS ON THE DEGREE OF UNSATURATION OF COW MILK FAT¹

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In a previous trial at this station, Frye (1) found that the environmental temperature to which cows were subjected was highly correlated with the iodine numbers of the milk fat they produced. In the light of these results a study was initiated to determine whether or not the great increase in milk fat unsaturation attending pasturing of cows results wholly from ingestion of pasture or is in part the result of higher temperatures characteristic of the grazing season.

Three lots of four cows were used. The four cows in each lot freshened at approximately the same time of the year, but each lot freshened at a different time of the year. This type of cow selection was employed with the hope that it might be possible to separate the effects produced by lactation from those that might result from temperature. Of each quartet, two cows were fed prairie hay and grain mixture (stall-fed) throughout the experiment; the other two were pastured and fed a grain mixture during the grazing season. The pasture group grazed at night only; the stall-fed group was placed in a drylot during the time the other group was on pasture in order to keep temperature effects as nearly alike as possible between the two groups. Both groups were kept in the barn during the day. Daily records of the environmental temperature were kept. Iodine and thiocyanogen values were determined on the milk fat resulting from a daily composite sample collected from each animal at approximately six-day intervals.

The data reveal that pasture and stage of lactation have primary influences on the degree of unsaturation of milk fat while changes in temperature appear to be of little importance.

Production of fats with a high iodine value at the peak of milk production followed by a decline in the iodine value (ca. 8 iodine units av.) to the fourth or fifth month of lactation and then a slight increase (ca. 3 iodine units av.) to the end of lactation appear to be definite lactation trends in the majority of the animals. These trends appear regardless of seasonal changes in temperature. The changes in iodine value were largely dependent on the changes in the oleic acid content of the milk fat. The percentage of linoleic acid decreased slightly in a linear manner during the lactation period.

Pasture feeding (measured in periods in which the effect of lacta-

¹ Doctoral thesis number 980, submitted July 13, 1943.

tion was at a minimum) increased the iodine value an average of 5 units. This increase was sustained throughout the period (in some instances 3 months) during which the cows were on pasture. When pasture was removed from the diet, the iodine value dropped rapidly to values that might be considered normal. Pasture feeding had little effect on the linoleic acid content of the milk fat. Changes in iodine value were largely dependent on the changes in the oleic acid content of the fat.

The high correlations between temperature and iodine value found by Frye can now possibly be explained as resulting from coincidence of lactation and temperature trends. A study of the freshening dates of the animals used by Frye indicate that this is likely.

A composite sample of milk fat was obtained from the cows in the college herd (approximately 120 cows) on the same dates that samples were collected from the experimental animals. The iodine value of the milk fat increased when the herd was pastured. This increase was not maintained. The iodine value gradually declined as the pasture season progressed. The herd cows were fed corn silage during the grazing period in addition to pasture. Since the six experimental cows which received pasturage but no silage during the grazing season maintained high iodine values throughout this period it was suspected that the decline in the iodine value of the herd sample during pasturage resulted from feeding corn silage. The possibility of decreasing summer milk fat unsaturation by feeding silage during the grazing season should be investigated.

In an attempt to obtain some information as to the cause of the high degree of unsaturation of milk fat resulting during pasturage and in early lactation, the following brief experiment was conducted. Thyroprotein was fed alternately to two cows to learn what effect an increased metabolic rate (resulting from thyroprotein feeding) would have on the iodine value of milk fat, since the metabolic rate of lactating cows is believed to be high during early lactation and during the pasture season. Thyroprotein caused the iodine value of the milk fat of one animal to be increased 6 units during the first 11 days of thyroprotein feeding. Thereafter the iodine value decreased and returned to the pre-thyroprotein level about 20 days later although thyroprotein continued to be fed. The increase in iodine value occurred during the period when the animal was losing body weight. The second cow was fed extra feed during thyroprotein administration. The iodine value of her milk fat increased 7 units after thyroprotein feeding. This increase was sustained for a period of 24 days (to the end of the experiment). This animal decreased in body weight during the first 14 days during thyroprotein feeding. However, the highest iodine values were found when the weight of the animal had stabilized. The extra feed supplied to this animal was thought to cause the iodine values to remain high. Since extra feed will increase the metabolic rate of cows fed thyroprotein (2) it was

thought that an increased metabolic rate of this animal was a factor responsible for increasing the iodine value of her milk fat.

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CERAMIC APPLICATIONS OF ZEOLITES ¹

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Synthetic zeolites have been used in the making of ceramic glazes but no actual comparison had ever been made or reported between glazes containing zeolite and glazes of the same identical chemical composition but made without zeolite. To make such a comparison, colored glazes were compounded using zeolites which had been treated with ion-bearing solutions, such ions being capable of producing ceramic colors. Other glazes were compounded whose ultimate melted compositions were identical with the zeolite bearing glazes, but in which were used feldspar and color-producing metallic oxides. All of these glazes received the same treatment and were fired to the same temperature for comparison. A cone 4 base glaze was used throughout.

0.60 PbO		
0.20 Na ₂ O	0.4 Al ₂ O ₃	2.4 SiO ₂
0.20 CaO		

The use of zeolite as a medium for carrying coloring ions in glazes which were never ground in a ball mill was also investigated. Zeolite and oxide-bearing feldspar glazes of the same composition were compared for advantages and disadvantages, both glazes being merely mixed in a high speed mixer, but neither was ground in a ball mill.

In an attempt to minimize or completely overcome some slight difficulties arising from the incorporation of zeolite into the glazes, mainly excessive shrinkage sometimes resulting in tearing and crawling, studies were made on the stabilization of zeolite. This was attempted both by use of heat treatment and by acid leaching. The temperature necessary to render the zeolite to such a state as to be unable to exchange ions was also determined.

A study was made of the effects of zeolite used as a deflocculant upon clay casting slips. These slips were compared insofar as pH and viscosity were concerned with slips of the same base composition and water content, but with sodium carbonate used as the electrolyte to

¹ Doctoral thesis number 1020, submitted December 14, 1949.

produce deflocculation. The composition used was that of a ceramic body previously found to be castable.

English China Clay	38
English Ball Clay	10
Feldspar	30
Whiting	2
Flint	20

A zeolite was prepared by leaching of sodium zeolite with hydrochloric acid until evidence of the aluminum in the leach water was found. After rinsing until chloride free, this material was found able to absorb sodium ions from dilute solutions. This material was added to over-deflocculated casting slips in an attempt to remove the excess sodium ions which produced the over-deflocculation. The sodium ions were present as a result of additions of sodium carbonate to the slip in order to produce over-deflocculation.

As a result of the work done to investigate the use of zeolite in ceramic glazes the following facts have been established:

1. The zeolite-bearing glazes were of excellent color, varying from pastels to darker shades. It was difficult, however, to establish with the means available that these glazes were superior in color intensity or color distribution to the glazes made in the conventional method.

2. The zeolite did materially aid in the melting of the glazes. At lower temperatures the zeolite glazes were less viscous than the feldspar glazes. At higher temperatures, since the compositions were identical, the melted glasses formed had equal properties for both types of glazes.

3. The zeolite glazes exhibited less pinholing and the presence of fewer bubbles than did the feldspar glazes fired to the same temperatures. This is a result of the higher rate of fusion afforded by the use of zeolite instead of feldspar.

4. Some of the feldspar glazes fired to lower temperatures had matte appearances indicating undissolved material, whereas the corresponding zeolite glazes fired to same temperatures had no or only slight matte appearances. This is also related to the ease of fusions.

5. The zeolite glazes had a greater range of firing temperatures than the feldspar glazes. This was exhibited by the fact that the feldspar glazes fired to lower temperatures were not completely in solution. This resulted in their becoming crazed after short periods of time. The zeolite glazes indicated no tendencies toward crazing.

6. The ease of crushing and grinding of the zeolite when the glaze was milled was beneficial in aiding the melting process. The extremely fine particle size of the zeolite did result in a tendency of some of the glazes to tear on drying and crawl on subsequent firing. This was minimized by correct application procedure and eliminated by sufficient heating of the zeolite prior to incorporation in the glazes.

7. Glazes of excellent color distribution and intensity can be pro-

duced, by use of zeolites, in glazes which have never been milled. Glazes made of feldspar and coloring oxides had speckled appearances, indicating poor color distribution. The advantage of the zeolite in this type of glaze is very apparent and could well lead to a new method of glaze formation.

Studies were made on the stabilization of zeolites from which the following conclusions were drawn:

1. Unwashed stock zeolite contains sodium carbonate which can interfere with the expected behavior of the zeolite. It is best removed by means of acid leaching with dilute acid.

2. All of the water of hydration of zeolite is completely removed by heating to a temperature of about $400^{\circ}\text{C}.$, most of it being lost prior to this temperature.

3. It is necessary that zeolite be heated to $950^{\circ}\text{C}.$ for at least 5 hours or to $1000^{\circ}\text{C}.$ for at least 3 hours to prevent the release of sodium upon subsequent grinding.

4. The use of dialysis as a means of removing the excess sodium ions present in freshly crushed zeolite is impractical.

Experiments were conducted on the use of zeolites in casting bodies. The following results are known:

1. Zeolite can be used satisfactorily and to an advantage as a deflocculant of clay casting slips. The zeolite is able to furnish the needed sodium ions and at the same time adsorb the undesirable calcium, magnesium, or other ions.

2. Casting slips deflocculated by zeolite have lower pH values for the same water content and viscosity than do the slips deflocculated by sodium carbonate.

3. The zeolite had a greater range of additions than did the sodium carbonate used for comparison. Five times as much zeolite as the theoretical minimum for deflocculation could be added. Increasing the minimum amount of sodium carbonate by one-third resulted in an over-deflocculated slip.

4. The treatment of zeolite with hydrochloric acid resulted in a zeolite capable of adsorbing sodium ions and lowering the viscosities of over-deflocculated slip.

5. The use of such a zeolite as mentioned (4) is governed by the particle size, the time allowed for reaction, and the amount of agitation or intimate mixing to insure complete ion exchange.

6. The use of a zeolite which can adsorb sodium ions should prove valuable in other fields as very few methods are available for the removal of sodium ions from solutions.

REACTIONS OF ORGANOMETALLIC COMPOUNDS WITH SOME BENZOTHAZOLE AND QUINOLINE TYPES¹

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The reactions of benzothiazole with organometallic compounds and the preparation of organometallic derivatives of benzothiazole have not been seriously investigated up to the present time, though Courtot and Tchelitcheff (1) have reported the metalation of benzothiazole by ethylmagnesium bromide. It was the purpose of this investigation to see if benzothiazole would form synthetically-useful organometallic derivatives and, also, if addition of organometallic compounds to the azomethine linkage of benzothiazole could be employed in the synthesis of 2-substituted benzothiazoles. In an attempt to explain some of the results obtained, certain 2-substituted quinolines were subjected to reactions with organometallic compounds. The literature review, therefore, was concerned with the activity of substituents, in the 2- and 4-positions of quinoline and pyridine, the 2- position of benzothiazole, and the 1- position of isoquinoline.

Benzothiazole was metalated in 90 per cent yield by *n*-butyllithium at -75° , and the resulting 2-benzothiazolyllithium was not stable above -35° (bath temperature). 2,2'-Bibenzothiazole was obtained in 44 per cent yield as one of the decomposition products. 2-Benzothiazolyllithium reacted with aldehydes, ketones, nitriles, and carbon dioxide to yield the expected products, but only a trace of 2-benzothiazolyl phenyl ketone was obtained from benzoyl chloride; no 2-benzylbenzothiazole was obtained from benzyl chloride; and no reaction was obtained with quinoline. Reactions which did not take place below -35° were complicated by decomposition of the 2-benzothiazolyllithium above this temperature. Under the same conditions that resulted in 90 per cent metalation of benzothiazole, quinoline gave an 84 per cent yield of 2-*n*-butylquinoline. Benzoxazole may have metalated under similar conditions, but attempts to detect the 2-benzoxazolyllithium by carbon dioxide and benzophenone, respectively, were unsuccessful.

Phenyllithium reacted with benzothiazole at 0° or above to yield some 2-phenylbenzothiazole. The yield was lower if 1 mole of phenyllithium was used than if 2 moles were used. The highest yield of purified 2-phenylbenzothiazole was 31.7 per cent. This was obtained from 2 moles of phenyllithium to 1 mole of benzothiazole at 0° for 1 hour. The reaction may have proceeded either by addition to the azomethine

¹ Doctoral thesis number 978, submitted July 8, 1949

linkage, if the phenyllithium added to the benzothiazole before metalation, or possibly by cleavage of the sulfur-carbon bond, if the reaction was between phenyllithium and 2-benzothiazolyllithium.

Phenyllithium reacted with 2-phenylbenzothiazole to yield bis-(*o*-aminophenyl) disulfide (7.70 per cent) and triphenylmethanol (8.36 per cent). This reaction probably involved both addition to the azomethine linkage and cleavage of the sulfur-carbon bond.

It was found that *p*-tolyl 2-quinolyl sulfide was cleaved at room temperature by phenyllithium to *p*-thiocresol and 2-phenylquinoline in good yield. This led to reactions of phenyllithium and other organometallic compounds (*n*-butyllithium, phenylmagnesium bromide, and phenylcadmium chloride) with other 2-electronegatively substituted quinolines (2-phenoxyquinoline, 2-ethoxyquinoline, 2-allyloxyquinoline, 2-benzoyloxyquinoline, 2-(*N*-piperidyl)quinoline, 2-chloroquinoline, and 2-benzylquinoline). In most cases, except with phenylcadmium chloride, some 2-phenylquinoline was obtained though the yields with phenylmagnesium bromide were low. Phenylmagnesium bromide did, however, react with 2-chloroquinoline to give a fairly good yield (31.2 per cent) of 2-phenylquinoline (2). Though *n*-butyllithium was the most reactive of the organometallic compounds, the yield of 2-*n*-butylquinoline was not so high as the yield of 2-phenylquinoline from phenyllithium because of more extensive side reactions. One of these side reactions resulted in 2-ethoxyquinoline-3-carboxylic acid, which was isolated from the reaction between 2-ethoxyquinoline and *n*-butyllithium followed by carbonation. The acid was identified by melting point, analysis, and conversion to 2-ethoxyquinoline-3-carboxamide (5), which was identified by a mixed melting point determination. Of the substituted quinolines, the 2-allyloxy- and 2-benzoyloxyquinolines showed the most side reactions. The 2-(*N*-piperidyl)quinoline was least reactive, and the 2-benzylquinoline, which was prepared from benzylsodium and quinoline, did not react. The reaction which results in cleavage may proceed by addition to the azomethine linkage or by a nucleophilic displacement.

Phenylcadmium chloride apparently gave a complex with quinoline, but, under the conditions of the experiment, did not form any 2-phenylquinoline. Phenylcadmium chloride did react with quinoline methiodide as does phenylmagnesium bromide to yield 58 per cent of *N*-methyl-2-phenyl-1,2-dihydroquinoline (4).

p-Tolyl 2-quinolyl sulfide (3) was prepared from 2-chloroquinoline and lithium thiocresoxide (80 per cent yield) and lead thiocresoxide (75 per cent yield), respectively. After crystallization from petroleum ether (b.p. 60–70°) the product melted at 68°.

2-(*N*-Piperidyl)quinoline was prepared from 2-chloroquinoline and lithium piperidide in 36.2 per cent yield and from 2-chloroquinoline and piperidine in 81.6 per cent yield. The melting point after crystallization from petroleum ether (b.p. 60–70°) was 51°.

The compounds prepared from 2-benzothiazolyllithium and aldehydes, ketones, and benzonitrile are listed below:

α -(*p*-dimethylaminophenyl)-2-benzothiazolemethanol (59.6 per cent yield) was recrystallized from benzene and melted at 157°; α -(*n*-propyl)-2-benzothiazolemethanol (55.7 per cent yield) melted at 82° after recrystallizing from petroleum ether (b.p. 77–115°); α,α -di-(*p*-dimethylaminophenyl)-2-benzothiazolemethanol (64.3 per cent yield) melted at 195° after recrystallization from benzene; α -methyl- α -(*p*-tolyl)-2-benzothiazolemethanol (62.9 per cent yield) was recrystallized from petroleum ether (b.p. 77–115°) and melted at 100.5°; α -phenyl-2-benzothiazolemethanol (66.2 per cent yield) melted at 123.5° after recrystallization from 95 per cent ethanol; α -methyl- α -phenyl-2-benzothiazolemethanol (56.0 per cent yield) was recrystallized from petroleum ether (b.p. 77–115°) and melted at 89°; α -(*p*-chlorophenyl)- α -methyl-2-benzothiazolemethanol (53.7 per cent yield) was recrystallized from 95 per cent ethanol and melted at 134–136°; α,α -diphenyl-2-benzothiazolemethanol (80.0 per cent yield) melted at 150° after recrystallization from absolute ethanol; di-(2-benzothiazolyl)phenylmethanol (61.5 per cent yield) melted at 150° after recrystallization from absolute ethanol; 2-benzothiazolyl phenyl ketone (71.1 per cent yield) was recrystallized from 95 per cent ethanol and melted at 102.5°. The phenylhydrazone of 2-benzothiazolyl phenyl ketone melted at 149°.

Some generalizations, based on the experimental work, were presented. One was concerned with the relative reactivities of organometallic compounds. The other generalization was concerned with the relative ease of replacement of the electronegative groups in the 2- position of quinoline.

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EFFECT OF HIGH SCHOOL SUBJECT PATTERNS UPON INITIAL ACHIEVEMENT IN THE CURRICULA OF THE DIVISION OF AGRICULTURE AT THE IOWA STATE COLLEGE¹

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The greatly increased enrollment during the past few years has brought students with a great variety of high school backgrounds into college. It was the purpose of this study to evaluate the various types of high school background submitted by the students for entrance into the Iowa State College Division of Agriculture. The evaluation was made in terms of the college achievement of the students with the various high school backgrounds. The criteria of achievement were (1) honor point ratio earned in first-quarter agriculture courses; (2) mark received in the initial course in chemistry; and (3) attrition-survival beyond the freshman year. No attempt has been made to define college achievement in terms of intangible benefits to the students such as personal satisfaction. Neither was there any attempt made to evaluate the various types of high school programs in terms of objectives of secondary education.

There were 997 male freshman students who entered the Iowa State College Division of Agriculture during the fall quarters of 1946, 1947, and 1948.

Transfer students, female students, and students for whom records were incomplete were eliminated from the study. The 997 students were classified into four high school pattern groups. All students who had taken three or more units of high school agriculture were designated as vocational agriculture high school students. All students who had taken six or more units of any combination of high school mathematics and science were designated as mathematics-science students. All students who had taken six or more units of high school vocational subjects other than agriculture were designated as other vocational students. All students with other combinations of high school background were designated as general pattern students. Because achievement in agriculture might be related to farm experience each of the foregoing groups was stratified into farm and nonfarm subgroups. The basis of the latter stratification was the father's occupation which the student reported.

For the purpose of making allowances for individual differences in

¹ Doctoral thesis number 1041, submitted March 13, 1950.

student ability, scores made on the American Council on Education Psychological Examination and first marks earned in the initial English course were used.

AGRICULTURE ACHIEVEMENT

Analysis of variance single classification, analysis of variance multiple classification with correction for disproportion among subgroups, and analysis of covariance single classification with American Council on Education Psychological Examination scores and English marks controlled were used to compare the agriculture achievement of the various groups and subgroups. No significant differences could be found in the achievement of the students in the various years. Students with farm backgrounds made significantly greater agriculture achievement than did students with nonfarm backgrounds. Significant differences in agriculture achievement were found among the high school pattern groups. When each high school pattern was compared with all others, the vocational agriculture group was found to have made greater agriculture achievement and the general pattern group was found to have made smaller agriculture achievement than had all other groups combined. The agriculture achievement of the mathematics-science group and the other vocational pattern group did not significantly differ from that of all other groups combined.

Within each high school pattern group the students with farm backgrounds were found to have made significantly better agriculture achievement that had the nonfarm students with the exception of the vocational agriculture pattern group. Within the vocational agriculture pattern group no significant difference in agriculture achievement could be found, between the farm and nonfarm background subgroups. To the degree that the various high school patterns can be evaluated in terms of first-quarter agriculture course achievement, the vocational agriculture pattern gave the best preparation and the general pattern the poorest preparation for college.

CHEMISTRY ACHIEVEMENT

Because the first course in chemistry could be taken at any time during the freshman or sophomore years the students who had entered college in the fall of 1948 had not all taken the first course in chemistry. Therefore, the 1948 group were excluded from the study of chemistry achievement. There was a total of 286 students who entered the Iowa State College Division of Agriculture during the fall quarters of 1946 and 1947 and had taken an initial chemistry course by the fall of 1949.

The first quarter of chemistry was reported as either 100A or 101. Chemistry 100A was the first course of a two-course sequence which dealt with the same subject matter as was presented in Chemistry 101. The marks reported were weighted for evaluation of achievement in an arbitrary 3 to 2 ratio as a compromise between considering quality

of achievement and quantity of material covered by the students in the two courses.

The weighted chemistry marks of the previously described groups and subgroups were compared by techniques similar to those employed in the evaluation of agriculture achievement. It was found that no significant differences in chemistry achievement could be demonstrated between the farm and nonfarm background groups. The farm and nonfarm groups were combined for subsequent analyses. When the chemistry achievement of the various high school groups was compared, significant differences were found. The chemistry achievement of the vocational agriculture, the other vocational and the general high school pattern groups was compared. No significant differences could be found. The previously noted differences in chemistry achievement were concluded to be associated with the mathematics-science group. A comparison of the mean weighted achievement of the various high school pattern groups corrected for differences in American Council on Education Psychological Examination scores and English marks revealed that the mathematics-science group made significantly greater chemistry achievement than did the other pattern groups.

To the extent that the various high school patterns can be evaluated in terms of weighted chemistry achievement, the mathematics-science pattern gave the best preparation for success. The other patterns did not differ from each other in the quality of preparation for achievement in initial college chemistry which they provided.

ATTRITION AND HIGH SCHOOL PATTERN

Of the 997 students included in this study, 606 were enrolled in the Iowa State College for the fall term following that in which they had entered as freshmen. The 391 students who failed to return were designated as the attrition group.

The differences among the various high school pattern groups in the proportions of students dropping out were made without control by chi square analysis in which expected numbers were derived from row and column totals. Using American Council on Education Psychological Examination scores and English marks as control factors, a modified discriminant function technique was used to derive expected numbers for a further chi square analysis. There were significant differences found among the high school pattern groups both with and without control. When an analysis was made on the attrition ratio among the vocational agriculture, other vocational and general pattern groups with the exclusion of the mathematics-science pattern group, no significant differences could be found.

In so far as the various high school pattern groups can be evaluated in terms of their attrition ratios, the mathematics-science group is apparently the most effective. The other three pattern groups appear to be equally effective.

AN ECONOMIC ANALYSIS OF PUBLIC LABOR POLICY¹

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The broad field of labor economics has been experiencing the pattern of expansion undergone by most other specialisms in the past. Numerous studies have been made dealing with labor legislation, trade-union behavior, unemployment insurance, wage theory, and labor problems during prosperity and depression. Although labor economists divide the field into rather arbitrary categories few include perhaps the most significant area: labor policy. This may at first seem odd, since the term labor policy appears frequently in the literature. This study, however, attempts to show that the term, as it is currently being used in the field, is misleading. A distinction should be made between industrial relations policy and labor policy. Industrial relations policy deals with the rights and duties of the parties in their contractual relations with each other and the extent to which government ought to intervene in the settlement of lawful industrial disputes. Labor policy concerns itself with the regulation and control of labor markets for the purpose of modifying the play of economic forces affecting the allocation of resources and the level of employment in the economy. It is the latter rather than the former area which this study intends to investigate.

Since society recognizes the need for policies dealing with periodic fluctuations in income and employment the major portion of this work addresses itself to the problem of integrating labor policy with the twin goals of full employment and continued economic growth.

The present study, therefore, attempts (1) to pull together the many investigations which have contributed to the area of labor policy as a bona fide subdivision of labor economics; (2) to explore the existing gaps in economic theory as they apply to questions of labor policy; and (3) to anticipate the major avenues of approach to be followed in the future.

Summarizing the findings of this work, we can make the following observations:

1. With relative equality of bargaining power between labor and management established, public policy must now concern itself with the economic consequences of wage-price determination on the part of trade unions and business firms.
2. Industrial relations policies are appropriate as a means of settling

¹ Doctoral thesis number 1002, submitted September 17, 1949.

industrial conflict. More important, however, are the independent actions of trade unions and business firms which do not give rise to conflict but manifest their effects on the level of employment and the allocation of resources.

3. The absence of public policy in this regard has compelled trade unions and business firms to deal with their own special problems through the process of adaptation.
4. The process of adaptation takes a number of forms designed to meet the following variety of circumstances:
 - a. Insecurity and uncertainty growing out of technological change.
 - b. Desire to maintain a particular job for the purpose of avoiding the problem of relocating in a new community, firm, or industry.
 - c. Desire to increase income, status, and prestige by means of restriction or the use of noneconomic codes of conduct.
 - d. Fear of suffering a reduction in income due to cyclical fluctuations in business activity and employment.
5. The cumulative effects of the process of adaptation as well as the rapid rate of economic progress are responsible for secular misallocations of labor.
6. Labor markets exhibit secular maladjustments with respect to supply in terms of regional variations in population growth and the disparity between actual increases in population and the rates of increase required for the maintenance of economic progress.
7. Secular maladjustments also appear on the side of demand and are largely a function of technological change and alterations in consumer's preferences.
8. With the development of new techniques, labor orientation becomes less of a factor in the determination of industrial location. Because of increased locational flexibility, secular labor policy must direct its attention toward narrowing the discrepancies between the regional demand and supply of labor resources by increasing the mobility of all factors of production.
9. The process of adaptation as well as secular labor market maladjustments can be overcome by remedying the periodic fluctuations in income and employment.
10. Recognition of full employment as a continuing policy of the national government has already been established. Unless an integrated public labor policy is also undertaken, no real solution for the problems already enumerated will be achieved. Moreover, serious difficulties, involving continuing inflation, will arise should labor policy be divorced from the goal of full employment.
11. Public labor policy must concern itself with two separate problems: (a) the income problem and (b) the resource problem. The political climate, however, will determine the means appropriate for each of these.
12. Integrating public labor policy with the goal of full employment, such that the optimum allocation of labor resources and stable

prices are achieved, can only be accomplished under economic totalitarianism.

13. Under relatively free collective bargaining a full-employment program will not mitigate the resource problem and continuing inflation will prevail.
14. With limited government intervention, arbitrary limits must be established with respect to permissible unemployment and price fluctuations. Such flexibility, however, reflects the price society must pay for maintaining some degree of freedom in the disposition of resources.
15. Although it may be useful, analytically, to assume the existence of separate political categories, in reality combinations of the political frameworks, mentioned above, may prevail. The acceptability of specific proposals in each category may be modified by the use of framework incentive planning and the principle of compensation.

ANALYTICAL STUDY OF DYNAMIC LOADS ON ELASTICALLY SUPPORTED SLABS¹

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Considerable work has been done during the past century on the general subject of analysis of plates. Expressions have been found for the deflection in plates of various shapes, these deflections having been caused by several different kinds of loading. The plates have been supported in diverse ways.

The present work was undertaken to study the effect of a dynamic load moving along a rectangular plate supported by an elastic foundation. The edges of the plate were pinned, that is, boundary conditions were imposed so that there was no deflection along the edges of the plate and the moments at the edges were zero. The initial conditions imposed were that at time $t = 0$ the deflection of the plate was zero and the plate was at rest.

The differential equation to be satisfied was

$$D \left[\frac{\partial^4 w}{\partial x^4} + 2 \frac{\partial^4 w}{\partial x^2 \partial y^2} + \frac{\partial^4 w}{\partial y^4} \right] + \rho w_{tt} + kw = f(x, y, t),$$

where w is the deflection, D the flexural rigidity, and ρ the mass density of the plate. The modulus of the foundation is designated by k . The function $f(x, y, t)$ represents the applied load.

Formal expressions for the deflection of an elastically supported rectangular plate of finite dimensions were found. First an expression for the deflection caused by a block load moving with a constant velocity was derived. This expression was changed, through a limiting process, to an expression that would give the deflection caused by a moving line load, and, from this, an expression for a moving point load was developed. No attempt was made to determine explicit values of the deflection in these three expressions.

An expression for the deflection caused by a point load moving with a constant velocity along a semi-infinite strip was next developed. This was done by letting the dimension a , the length of the plate in the x -direction, increase without limit. The expression for the deflection caused by this point load was expressed as a double integral.

The first of these integrations was performed using Cauchy's theorem of residues. The expression was then broken down into three terms. The location of the poles of the integrands, in the second integra-

¹ Doctoral thesis number 1013, submitted December 12, 1949.

tion, depended upon the velocity at which the point load was moving. Five distinct cases were considered. Only the steady state deflections in the neighborhood of the load were wanted, that is, the deflections near the load when $t \rightarrow \infty$. The saddlepoint method was used to evaluate these integrals as this method was applicable for large positive values of time. Cauchy's theorem of residues was again used. The location of the saddlepoint was found to be a function of x . As the contour of integration always passed through the saddlepoint, several different variations as to the number of poles falling within the contour were found. This caused each of the three terms to have several different values. These values depended upon the velocity at which the load was moving and also upon the location of the point, with respect to the load, at which the deflection was to be calculated. Different combinations of these three terms gave the proper deflection expressions.

The final results express the deflection functions as infinite series. These functions approach validity as time increases indefinitely, that is, only after the load has been in contact with the plate for a long period of time.

BONE DARKENING IN FROZEN POULTRY¹

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This was a study of the factors involved in bone darkening of frozen poultry. The methods of processing, freezing, storing, thawing, and cooking were investigated to determine their effect on the discoloration of the bones. Attempts were made to elucidate the causes of bone darkening and to find means of preventing its occurrence.

Twelve-week-old New Hampshires of both sexes were used for most of the experiments. A few mature birds were also observed. After freezing, storing, and thawing, the birds were cooked and the meat was removed from the bones. The extent of bone darkening was measured subjectively by a panel of four judges using for comparison standard bones that were selected to represent scores of 0 to 10. A bone without appreciable color was scored 0.

It was demonstrated that freezing and thawing hemolyze the red blood cells of the bone marrow. The hemoglobin thus liberated penetrates the walls of the bones, causing discoloration. However, when the bone marrow erythrocytes were hemolyzed without freezing (by injecting a 2 per cent saponin solution into the bones) no darkening resulted. It was indicated, therefore, that freezing and thawing not only liberate the hemoglobin in the bone marrow but also make it possible for the hemoglobin to penetrate to the outer surface of the bone.

Examination of unfrozen and frozen, defrosted fryers revealed that all bones were somewhat darkened by freezing and thawing but the leg, thigh, and coracoid bones were the most seriously affected. In the raw state, frozen, defrosted bones were shown to be red in color but when cooked the color changed to a reddish brown. Freezing and thawing did not darken the bones of yearling New Hampshire hens.

It was found that the conditions of freezing and storing did not influence bone darkening sufficiently to be of practical importance. The factors studied included freezing rate, temperature of storage, length of the storage period, and temperature fluctuations during storage.

The effects of various processing methods on bone darkening were observed. The extent of bleeding, the degree of carcass separation, and the chilling method did not affect the color of the bones of frozen birds. Refreezing defrosted stock was not found to produce increased bone darkening. Covering the disjointed carcasses with water or solutions

¹ Doctoral thesis number 995, submitted August 20, 1949.

of NaCl, ascorbic acid, or KNO_3 prior to freezing did not reduce the extent of bone discoloration.

New Hampshires between the ages of 7 and 14 weeks did not differ significantly with respect to bone darkening when frozen. At the age of 16-17 weeks, however, bone color scores were reduced. On the basis of the bone darkening observed in various breeds and crossbreds, it was concluded that the genetic background of the birds is not an important factor.

Removing the bone marrow from the leg and thigh bones prior to freezing prevented bone darkening. A practical method for removing bone marrow was proposed. The failure of the bones to darken after removal of the bone marrow, coupled with the fact that poor bleeding did not increase bone darkening, indicated that the bone marrow is the only source of the pigment discoloring frozen bones.

Different methods of cooking (i.e. frying, boiling, and roasting) were not found to differ greatly with respect to the development of bone discoloration in frozen birds. However, roasting the pieces of chicken 30 minutes or more prior to freezing reduced the bone color scores considerably.

Rapid thawing was accomplished by placing the frozen birds in the oven to cook without prior defrosting and by immersing the packages in water at 140°F . Either of these procedures prevented the development of dark bones provided that the birds had been frozen and stored at -30°F . and were cooked immediately after thawing. Rapid thawing did not prevent bone darkening when the birds had been frozen and stored at -10°F . nor when cooking was delayed for 72 hours after thawing.

TECHNIQUE FOR EVALUATING THE ABILITY OF TEACHERS TO APPLY PRINCIPLES CONCERNED WITH THE DEVELOPMENTAL NEEDS OF ADOLESCENT GIRLS¹

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The purpose of this study was to develop a technique for evaluating the ability of secondary school teachers to apply the principles concerned with the developmental needs of adolescent girls. The first hypothesis was that pencil-and-paper tests could be developed which would measure the ability of teachers to recognize and apply principles concerned with human growth and development of adolescents. Two tests were developed to measure this ability: Test A, Recognition of Principles, and Test B, The Case of Jacqueline Croner, essay and short-answer forms. The second hypothesis was that one case study would be sufficient to measure this ability.

The assumption was that it is important for all teachers to be able to apply principles concerned with the developmental needs of children. The experimental group in the study was composed of home economics teachers at the secondary level, on the further assumption that instruments which were valid and reliable for that group would be equally useful for all secondary school teachers.

Test A, Recognition of Principles, was made up of principles which related to the needs of adolescents in three major aspects of development: physiological, social, and integrative.

Test B, The Case of Jacqueline Croner, was designed to measure the ability which teachers have to apply principles in interpreting data concerned with the developmental needs of adolescents, and to select educational means for helping pupils with their problems. The test was a description of an adolescent based on data from a cumulative record of a high school girl.

Tests A and B were revised after having been administered to a preliminary group of thirty-five home economics teachers to discover any lack of clarity in vocabulary and statements, to discover difficulties in administration, and to determine the length of time required to give the test. The revised tests were administered to juries of four to six judges, and from their responses the keys for scoring were determined.

The group to whom the tests were then administered was made up of eighty-eight home economics teachers in West Virginia who had signified a willingness to participate. Several factors were considered in

¹ Doctoral thesis 1005, submitted November 2, 1949.

selecting these teachers: marital status, recency of graduate study, length of teaching experience, and the feasibility of visits by the writer.

Test B, The Case of Jacqueline Croner, essay form, was sent to each of the participants. This was followed by Test B, short-answer form, and Test A, Recognition of Principles.

The reliabilities of Tests A and B were determined by obtaining the correlation coefficient between scores on split halves. A Pearsonian coefficient of correlation was obtained, .73 and .840 respectively, and when corrected for double length by the Spearman-Brown modified formula became $.848 \pm .030$ and $.913 \pm .018$.

Two methods for establishing validity of the instrument were employed. One was the correlating of scores on Test A and on Test B, essay form, as well as the scores on Test A and Test B, short-answer form. The coefficients obtained were:

Test A and Test B, essay	$.955 \pm .009$
Test A and Test B, short-answer	$.976 \pm .005$
Test B, short-answer, and Test B, essay	$.936 \pm .013$

In order to have greater confidence in the results, an additional group of ninety home economics teachers in West Virginia took tests A and B. The procedure except for visits and interviews was duplicated and the coefficients of correlation were again obtained.

The coefficients for the second group were:

Test A and Test B, essay	$.951 \pm .010$
Test A and Test B, short-answer	$.956 \pm .009$
Test B, short-answer, and Test B, essay	$.925 \pm .015$

These results would seem to indicate that the high correlations of the first group were not due to sampling, since the differences in the correlations are slight.

A second method of validation used was that of computing coefficients of correlation between the performance on the tests and a criterion assumed to be valid. In this study that criterion was the scores obtained on interviews which the writer had with the teacher, the principal and/or the superintendent and the score on a 1-day observation of the teacher, designated as an interview-observation score. This score was obtained from information relative to how the teacher applied principles concerned with the developmental needs of adolescent girls in a real-life situation. It was determined by the use of a scale which had a range of 1 to 15. When coefficients of correlation were computed between the scores on the tests and the interview-observation scores, the correlations were:

Test A and interview-observation scores	$.875$
Test B, essay, and interview-observation scores	$.901$
Test B, short-answer, and interview-observation scores	$.911$

Since Test B was designed to measure the ability of the teacher to

diagnose problems and select remedial measures, coefficients of correlation to determine whether one section of the test was more valid than the other were computed. The correlation between the diagnostic sections on the two forms of Test B was $.788 \pm .040$ whereas the correlation between the scores on the two remedial sections was $.433 \pm .087$. Also computed were coefficients of correlation between the scores on the remedial section of the short-answer form of Test B and the interview-observation scores; the result was a coefficient correlation of $.787 \pm .041$.

The items on both Test A and Test B were analyzed to determine internal consistency and to discover items which should be discarded or revised because they were not discriminating. All except 13 of the 62 items in Test A and 30 items of the 110 in Test B were satisfactorily discriminating.

In a teacher-education program it would be valuable if devices which could be administered easily were available, so that a prediction of what a teacher would do relative to the application of principles when she is faced with a real-life situation could be made. In the present study paper-and-pencil tests have been developed which appear to be valid and reliable, hence can be used as the independent variables in computing regression equations. For the criterion, the interview-observation score, which was used as the dependent variable in determining regression, the validity was assumed.

There were three main problems to be considered in analyzing the regression data. Can a regression equation which will predict teacher behavior in a real-life situation with reasonable accuracy be determined? How accurate would the prediction be if only the short-answer tests, X_1 and X_2 , were used? How much better is the interview-observation technique than the combination of written tests?

In the following discussion of regression, the scores on the two tests and the interviews and observations will be designated by these symbols:

X_1 = Test A: Recognition of Principles Test

X_2 = Test B: The Case of Jacqueline Croner (short-answer form)

X_3 = Test B: The Case of Jacqueline Croner (essay form)

Y = Interview-observation.

The first problem was to set up regression equations. The equation using all three variables is:

$$Y = -6.0096 - .2235X_1 + .2556X_3 + .2517X_2$$

The equation using two variables is:

$$Y = -.0741X_1 + .2644X_2 - 5.9562$$

The equation using one variable is:

$$Y = 2140X_1 + 1.9382$$

To ascertain the usefulness of these equations it was necessary to determine the multiple coefficients obtained when all variables were used or progressively dropped; to determine the standard partial regression correlations holding various ones of the variables constant; and

to make tests which indicate the significance of regression coefficients.

The multiple correlation coefficient obtained between all the estimated Y values and the actual values was .9366.

The multiple R^2 is .8773 which means that 87.73 per cent of the variability among teachers in their ability to apply principles in this area can be measured by the three pencil-and-paper tests. If Test B, X_3 , is omitted from the multiple correlations, R^2 becomes .8394. If this independent variable is dropped from the equation, an additional 4.24 per cent of the variability remains unaccounted for by regression.

The standard partial regression coefficient specifies changes in Y , independent of the changes in the other independent variables. The b' for each of the three tests is:

$$X_1 = .9139$$

$$X_2 = 1.1492$$

$$X_3 = .6984$$

It is obvious that X_2 is the most important test in predicting Y .

Standard partial regression coefficients may also be used to judge which of the independent variables are most important in estimating Y . If X_2 is held constant and X_1 is compared with Y , the correlation is low, .0160. If X_3 is held constant and X_1 is compared with Y the correlation is low, .1071.

If X_1 is held constant the correlations between X_2 and Y and between X_3 and Y are:

$$r_{Y\ 2.1} = .5449$$

$$r_{Y\ 3.1} = .4577$$

When X_3 is held constant the correlations between X_1 and Y , between X_2 and Y , and between X_1 and X_2 are:

$$r_{Y\ 1.3} = .1071$$

$$r_{Y\ 2.3} = .4431$$

$$r_{12.3} = .7840$$

When X_1 is held constant, the correlation between X_3 and X_2 is:

$$r_{32.1} = .0619$$

When each independent variable is compared with Y , the other two variables being held constant, the following correlations are obtained:

$$r_{Y\ 1.23} = .4318$$

$$r_{Y\ 2.13} = .5818$$

$$r_{Y\ 3.12} = .5495$$

Since all t tests coefficients 5.385, 6.559, and 4.385 are highly significant, each of the independent variables may be assumed to be related to the dependent variables in a linear manner.

Throughout the statistical analysis X_3 , the essay form of Test B, The Case of Jacqueline Croner, proved to be the least valuable of the variables to use for prediction purposes. It had the smallest standard partial regression coefficient, the smallest partial correlation with the Y variable, and the smallest t value. It seems, therefore, that it is justifiable to drop

it from the regression equation and lose a very small part of the total predictable information in favor of more easily administered and time-saving tests.

The recommended regression equation obtained is:

$$Y = -.0741X_1 + .2644X_2 - 5.9562$$

It can be reasonably assumed that the ability of individuals to apply principles can be measured by the use of X_1 and X_2 . These tests should make it unnecessary for teacher-educators to expend time, energy, and money in visiting widely distributed schools for the purpose of observing teachers in their classrooms and during extra-curricular activities, of interviewing them and consulting their administrators in order to determine their ability to apply principles in this area.

CONTROL OF ASPHALTIC CONCRETE MIXTURES AT HOT-MIX PLANTS¹

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Asphaltic concrete pavements have been extensively used for primary roads during recent years. Most of them belong to the hot-mix, hot-laid type. Since the knowledge about asphaltic concrete mixtures is not so complete as that regarding other common engineering materials, there are still many uncertainties involved in the design, manufacturing, and laying of such mixtures. Consequently, every step in asphaltic paving deserves critical attention. This study covers the control of the mixture at hot-mix plants.

By reviewing the process in manufacturing asphaltic concrete mixtures, it is seen that there are many hard to control factors which affect the quality of output. If there is no rapid plant test to indicate quality of mixture, the plant inspector cannot assure that the output is always satisfactory.

A survey of prevailing inspection practices reveals that the desired rapid quality-indicating test is not available now. Many states depend too much upon personal experience of engineers and inspectors; others specify certain laboratory stability tests as a control for plant operation. Although personal experience is helpful in all constructional works, some definite control test seems to be more desirable. On the other hand, stability tests performed at the central laboratory are usually not so effective for plant control because there is too much delay for the delivery of sample and report. Laboratory tests are good only for preliminary design and occasional check. In order to facilitate immediate control at asphaltic concrete plants, it is necessary to have a rapid quality-indicating test which can be conveniently applied at the plant.

The main objective of this research project is to develop the desired rapid quality-indicating test. The term Plant Stability Test will be used for this test device. Plant Stability Test is empirical in nature, but every effort has been made to simulate the actual loading under service conditions. Although this test involves plastic properties of asphaltic concrete mixtures, only the compressive strength of the specimen is recorded. Compressive strength of the specimen is considered as a measure of resistance to deformation. Previous studies in the field of asphaltic concrete paving have shown that resistance to

¹ Doctoral thesis number 1000, submitted August 24, 1949.

deformation of any asphaltic mixture is affected by type and gradation of aggregate (including filler), type and content of asphalt binder, density of mixture, etc. Therefore, the first step for investigating the effectiveness of this test was to verify its sensitivity to all these factors. Experiments indicated that the suggested test had a high degree of sensitivity.

In order to have some idea about the relationship between this test and the more common mechanical tests, a comparative study has been made. Specimens prepared with similar compactive effort were tested by Hveem Stabilometer, Hubbard-Field, Marshall, Unconfined Compression and the suggested method. Results disclose that the Plant Stability Test is as effective as an indicator of quality as any of the other tests.

The most unique feature for the Plant Stability Test is the elimination of a constant temperature oven or water bath used for heating the specimen before testing. Since it is absolutely necessary to test specimens at a definite temperature, a technique has been developed in which an armored thermometer is inserted in the specimen to indicate temperature at the time of test. Various experiments have been performed in order to verify the dependability of this procedure. By controlling the temperature for both the mold and mixture just before molding, it is possible to get a positive temperature control for the specimen during testing.

Another feature of the suggested test is the use of the same cylindrical mold for both molding and testing. By such a device, time and labor for extrusion of the specimen from the mold after molding can be saved.

Compactive effort for the preparation of the specimen has been studied by laboratory experiments correlated with actual field conditions. It is recognized that density alone cannot represent the efficiency of consolidation. Orientation of aggregate particles plays an important role in establishing the stability of any compacted mixture. Since there is a definite tamping action in modern asphaltic concrete paving machines, a dynamic load furnished by a 10-pound hammer is applied to the specimen before the compaction by static load. It is intended to simulate the actual efficiency of consolidation by such a combined compactive effort.

Other laboratory investigations in regard to the test specimen include temperature of mold, height of specimen, etc. All laboratory experiments act as experimental backgrounds for the development of Plant Stability Test.

As Plant Stability Test was designed for the use at hot-mix plants, it must be actually applied at the plant in order to verify its practical value. A portable testing machine and other equipment were built for this purpose. Results indicate that this test can be very conveniently applied for plant control. The principal testing procedure is outlined as follows:

Weighing—Proper amount of hot mixture is weighed out in an insulated pan. A thermometer inserted in the pan indicates the temperature of mixture.

Molding—Molding of specimen must be started when temperature of mixture drops to 260°F. The specimen receives fifty blows by a 10-pound hammer dropping from a height of 18 inches. Then, a static load of 1,000 pounds per square inch is applied for 1 minute.

Testing—An armored thermometer is attached to the compression ram which has been placed over the specimen during molding. This thermometer measures the temperature at the center of the specimen. When the thermometer reading drops to 160°F., the specimen is tested right in the mold with removable walls taken away. The compressive strength of the specimen is termed the stability value of the mixture.

Time from the beginning of molding to the end of testing is approximately 10 minutes. With such a rapid control test, a plant inspector knows the quality of mix. He can make any necessary adjustment of the mix if the stability value found from this test is below the minimum requirement. By applying the suggested test at hot-mix plants, effective control of asphaltic concrete mixture will not be difficult.

FACTORS ASSOCIATED WITH PERFORMANCE
OF 4-H CLUB VOLUNTEER LEADERS IN
NEW YORK STATE, 1946-48¹

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This abstract summarizes a study of Factors Associated with Performance of 4-H Club Volunteer Leaders in New York State, 1946-48. The study was designed to analyze some of the social, group, and cultural factors influencing adult volunteer leaders as they function in a group situation in rural communities.

Many studies have been conducted covering various phases of 4-H Club volunteer leadership. The highly significant area of factors which explain satisfaction and its relationship to performance and tenure needs intensive study if problems of selection, training, and recognition of volunteer leaders are to be better understood.

The purposes of this study are: (1) to explain varying degrees of satisfaction among adult volunteer leaders associated with the 4-H Club program; (2) to determine the relationship of satisfaction to such criteria as success, performance, and tenure; (3) to suggest areas for further research; and (4) to set forth certain suggestions and recommendations, based on the findings and conclusions of this investigation, that would aid professional workers in developing a more effective program.

Information was obtained from 760 club leaders located in twelve counties of New York State. A pretested schedule was used by trained interviewers in obtaining the basic data.

Leaders were divided into two groups—those currently active (present leaders) numbering 510, and those who had discontinued their leadership duties (past leaders) of which there were 250. Leaders were classified by the use of attitude scale analysis into five scale types of satisfaction, representing the range from the most satisfied to the least satisfied on a continuum. Four scale types of success were established based on opinion ratings of 4-H Club Agents and the interviewers. Three levels of performance—high, medium, and low—were developed on the basis of numerical performance of the clubs with which the leaders were associated. Each of the three criteria of satisfaction, success, and performance was related to the other by the use

¹ Doctoral thesis number 1045, submitted May 19, 1950.

of chi-square. The three criteria were combined into a composite rating termed *desirability*.

Within the limitations of the criteria for measuring satisfaction, success, and performance and the statistical methods used in this study, the following tentative conclusions may be offered:

1. Scale analysis provides a statistical model and an objective procedure for approaching the problem of measuring attitudes of satisfaction and success. Scale analysis does not, however, define the attitude or what constitutes success.

2. One can conclude that the association between satisfaction, success, and performance of the 760 leaders studied is so marked that we cannot believe the three criteria are not associated in the universe of 4-H Club leaders from which our sample was drawn.

3. In general the association between satisfaction, success, and performance was more pronounced for present than for past leaders.

4. There was a marked association between satisfaction, success, and performance as expressed by 4-H Club leaders and such measurable personal factors as age, education, income of the household, social participation, and occupation. The number of years of operation of the 4-H Club with which the leader was associated, the number of years the person had been a 4-H Club leader, the kind of recognition received, the number of training meetings the leader had attended, and the degree of importance the leader placed on prizes and awards received by his club members were also significantly associated with scale types of satisfaction and success and levels of performance.

5. Factors which did not appear to be significantly associated with these three criteria were: marital status, place of residence, type of 4-H Club, the number of persons in the leader's household, whether or not there were 4-H Club members in the leader's household, and the amount of recognition which the leaders had received.

6. When the interdependence of such factors as tenure, occupation, education, and participation were determined in relation to desirability, it was found that: (a) participation and tenure appeared to be associated with the desirability of persons as 4-H Club leaders; (b) the farmer and homemaker occupational grouping also appeared to be indices of desirability; (c) the professional, white collar, teacher, and student occupational grouping did not appear to make for desirability as 4-H Club leaders; (d) persons with twelve grades of schooling or less appeared to be more desirable as 4-H Club leaders than did individuals with college and technical training.

7. Recognition to the leaders in the form of interest and appreciation shown by 4-H Club members, their parents and people of the community was a more effective means of motivation than that provided by awards, banquets, publicity, and trips.

8. Four-H Club leaders who discontinued their leadership duties after 1 to 3 years were, in general, younger, males rather than females,

had less formal schooling, single rather than married, had lower incomes, and were less active in community organizations and agencies than were leaders who continued to assume their responsibilities for more than 3 years.

Additional areas for research suggested by this particular study are the interrelationship of such factors as tenure, education, occupation, and participation, as criteria for predicting success and performance of 4-H Club leaders; the "halo" effect of self evaluation and rating of others; the leadership role of an adult in relation to the social, group, and cultural patterns of the community in which the club is located; factors which serve to effectively motivate leaders; and the role of the professional leader (4-H Club Agent) in relation to volunteer leaders.

Some of the implications that can be drawn from this study are as follows: (1) The selection of 4-H Club Leaders should be largely the responsibility of the community concerned rather than the 4-H Club Agent. The former procedure helps to identify the leader and his responsibilities with the needs and support of the community. (2) Assuming the goal is to choose leaders who will achieve the maximum satisfaction and performance, one should emphasize those personal and group factors which this study showed are significantly related to these important criteria. (3) The 4-H Club Agent will find it advantageous to have more direct, face-to-face contact with club leaders. Leaders desire personal counsel and encouragement on many problems arising in the early stages of their leadership duties. (4) Every effort needs to be directed toward sensitizing the parents of club members, the members themselves, and people of the community to the need for, and importance of, expressing verbal and written approval to the 4-H Club leader for what he is doing.

RELATIONSHIP OF THE NUTRITION OF *STREPTOCOCCUS LACTIS* TO BACTERIOPHAGE PROLIFERATION¹

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Evidence now exists that bacteriophage reproduction, although related very closely to bacterial reproduction, may be influenced separately under certain nutritional conditions. This investigation offers information concerning the relation of the multiplication of *Streptococcus lactis* to bacteriophage multiplication in various chemically defined media.

The chemically defined medium reported by Niven (1) was used as a basis for the study. Turbidity measurements were used as an indication of organism growth, and the numbers of bacteriophage particles were determined by the limiting dilution method in litmus milk. Three samples of each dilution were made, and the most probable number of bacteriophage particles per milliliter was computed using probability tables. An incubation temperature of 32°C. was used for all experiments.

The synthetic medium of Niven was found to support the growth of twenty-two of thirty-one strains of the *Streptococcus lactis* type, but it did not support the growth of any of twenty-two strains of the *Streptococcus cremoris* type. Since homologous bacteriophages were not available for nineteen of the twenty-two strains of *S. lactis* which grew on the synthetic medium, work was conducted using only three *S. lactis*-bacteriophage combinations. Bacteriophages active against two of these three strains of *S. lactis* were found to multiply readily on their respective host cells in the synthetic medium. The most probable number of bacteriophage particles usually increased from about one thousand to about one million per milliliter during a 7-hour incubation period. While the third strain of *S. lactis* grew readily on the synthetic medium, the homologous bacteriophage did not multiply.

In a study to determine the effect of the omission of individual components from the medium upon the multiplication of the two *S. lactis*-bacteriophage combinations, each amino acid, vitamin, and purine or pyrimidine base was omitted individually from the complete medium. When the omission of an individual component from the medium caused a decrease in bacteriophage multiplication, a parallel decrease in bacterial growth also was encountered. Bacteriophage multiplication seemed to be very closely related to bacterial multiplication for these two combinations.

¹ Doctoral thesis number 981, submitted July 13, 1949.

A study was made to determine the nutrients necessary for the growth of other strains of the lactic group in the synthetic medium. When small amounts of sodium acetate and Tween 80 were included in the synthetic medium, turbidity was developed by the twenty-two strains of the *S. cremoris* type and the nine strains of the *S. lactis* type which did not grow in the medium of Niven. Those strains of the lactic group which did not require sodium acetate and Tween 80, a source of oleic acid, were stimulated when these nutrients were present. Neither sodium acetate nor Tween 80 alone would suffice for serial transfer of many cultures; however, sodium acetate alone was effective for more cultures than was Tween 80 alone. The requirement of strains of the lactic group for sodium acetate and Tween 80 could not be modified by a mixture of several growth factors which have been reported important in the nutrition of various lactic acid bacteria. The liver extract, reticulogen, however, could substitute, in comparatively much smaller amounts, for sodium acetate and Tween 80. In addition, reticulogen stimulated the growth of one strain of the *S. cremoris* type which did not become turbid until after 24 hours when the supplement was sodium acetate and Tween 80.

While the synthetic medium supplemented with sodium acetate and Tween 80 supported the growth of all organisms used, it was inadequate for the multiplication of bacteriophages on the nine *S. cremoris* strains which were used in this portion of the study. Even when supplemented with a mixture containing all of the known vitamins of the B complex, including B₁₂, and several other known growth factors, the bacteriophages did not multiply. Such complex mixtures as yeast extract, tomato juice, proteose peptone, reticulogen, peptonized milk, and hydrolyzed casein were used to no avail. Even the addition of 5 per cent dried skimmilk did not make the medium adequate for the multiplication of bacteriophages until potassium phosphate was left out of the medium. It then was discovered that when calcium was available, the completely synthetic medium was adequate for the multiplication of all of the bacteriophages which would not multiply on the medium as used previously. In the synthetic medium supplemented with 0.1 per cent calcium chloride, the precipitation of tricalcium phosphate was avoided by decreasing the potassium phosphate content to 0.1 per cent and by either sterilizing the complete medium by filtration or autoclaving the potassium phosphate separately from the other components. When calcium was available in the medium, the close relationship between multiplication of bacteriophage and organism was again evident. The addition of calcium had no detectable effect on organism growth.

An adsorption study indicated that calcium possibly was not necessary for the adsorption of bacteriophages to bacterial cells. However, the accuracy of estimation attained in enumerating the bacteriophage particles leaves considerable question as to the validity of this conclusion.

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I. IONIZATION AND HYDRATION EQUILIBRIA OF PERIODIC ACID ¹

II. SOLUBILITY AND COMPLEX ION FORMATION OF THE RARE EARTH OXALATES

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I. IONIZATION AND HYDRATION EQUILIBRIA OF PERIODIC ACID

A study of the ultraviolet absorption spectra of periodic acid as a function of the hydrogen ion activity, the activity of water, and the temperature revealed that the optical density is a function of all these quantities. Also it was found from potentiometric data that the ionization constants of periodic acid had unusually large variations with temperature changes, becoming a stronger acid at higher temperatures. In addition, it was found that the pH of a solution of periodic acid decreased as the ratio of methanol to water in the solvent was increased. Thus, periodic acid appeared to be a stronger acid in methanol-water mixtures than in pure water. All of the above observations have been quantitatively explained by postulating that periodic acid exists in solution mainly as a mixture of two acids, the relatively weak paraperiodic acid, H_7IO_6 , and the very strong metaperiodic acid, HIO_4 . From the ultraviolet absorption data and the potentiometric studies, the following equilibria have been evaluated in a temperature range of 0–70°C.:

$$(1) \quad \frac{(\alpha_{\text{H}^+}) (\alpha_{\text{IO}_4^-} + \alpha_{\text{H}_7\text{IO}_6^-})}{\alpha_{\text{H}_7\text{IO}_6}} = K'_1$$

$$(2) \quad \frac{(\alpha_{\text{H}^+}) (\alpha_{\text{H}_7\text{IO}_6^-})}{(\alpha_{\text{IO}_4^-} + \alpha_{\text{H}_7\text{IO}_6^-})} = K'_2$$

$$(3) \quad \frac{(\alpha_{\text{H}^+}) (\alpha_{\text{H}_7\text{IO}_6^-})}{\alpha_{\text{H}_7\text{IO}_6}} = K_1$$

¹ Doctoral thesis number 1070, submitted June 5, 1950.

$$(4) \quad \frac{(\alpha_{H^+}) (\alpha_{H_4IO_6})}{\alpha_{H_4IO_6^-}} = K_2$$

$$(5) \quad \left(\frac{\alpha_{IO_4^-}}{\alpha_{H_4IO_6^-}} \right) a_{H_2O}^2 = K_D$$

where the above constants are related by the following:

$$(6) \quad K'_1 = K_1 (K_D + 1)$$

$$(7) \quad K_2 = K'_2 (K_D + 1).$$

II. SOLUBILITY AND COMPLEX ION FORMATION OF THE RARE EARTH OXALATES

The solubility of cerium, neodymium, and ytterbium oxalates has been studied by employing radiotracer techniques in various oxalate-buffered solutions at 25°C. The solubility data gave fairly smooth functions when correlated with the $\alpha_{C_2O_4^{2-}}$, and attempts to include the $\alpha_{HC_2O_4^-}$ in the explanation of the experimental values failed to reveal any primary effect in controlling the solubility of the rare earth oxalates. Variations in the α_{H^+} were only effective in controlling the oxalate activity as calculated from the ionization equilibria and the total oxalate in solution.

$$(1) \quad \alpha_{C_2O_4^{2-}} = \frac{K_1 K_2 M}{\alpha_{H^+}^2 + \frac{\alpha_{H^+} K_1}{\gamma_1} + \frac{K_1 K_2}{\gamma_2}}$$

where M = total oxalate in solution

K_1 and K_2 = first and second ionization constants of oxalic acid.

Calculation of the oxalate ion activity was accomplished by means of successive approximations in which the ionic strength, and hence the activity coefficients, from each preceding computation were employed.

The possibility of ions of the type $R(C_2O_4)_n^{3-2n}$ was considered, where R is the rare earth ion. The slope of the curves representative of the data in the region of high oxalate ion activity indicated the complex species present in this region, since to a first approximation the formation of $R(C_2O_4)_2$ would lead to a slope of + 3/2, $R(C_2O_4)_2^-$.

to a slope of $+1/2$, and $R(C_2O_4)^{\cdot-}$, to a slope of $-1/2$. The following equilibria explained the experimental data of the three rare earth systems studied:

- (2) $R(C_2O_4)_3^{\equiv} \rightleftharpoons R(C_2O_4)_2^- + C_2O_4^{\equiv}; K_I$
- (3) $R(C_2O_4)_2^{\equiv} \rightleftharpoons R(C_2O_4)^+ + C_2O_4^{\equiv}; K_{II}$
- (4) $R(C_2O_4)^+ \rightleftharpoons R^{+++} + C_2O_4^{\equiv}; K_{III}$
- (5) $R_2(C_2O_4)_3(s) \rightleftharpoons R^{+++} + R(C_2O_4)_3^{\equiv}; K_{sp_1}$
- (6) $R_2(C_2O_4)_3(s) \rightleftharpoons R(C_2O_4)^+ + R(C_2O_4)_2^-; K_{sp_2}$
- (7) $R_2(C_2O_4)_3(s) \rightleftharpoons 2 R^{+++} + 3 C_2O_4^{\equiv}; K_{sp_3}$

The above six equilibrium constants were evaluated at 25°C. from the data.

THE EFFECT OF ROOFING MATERIALS ON TEMPERATURES IN FARM BUILDINGS¹

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This study consisted of an analysis and an experimental study of the effect of roofing materials on temperatures in farm buildings under summer and winter conditions. It was a part of the over-all study which is being carried on by the Iowa Agricultural Experiment Station to determine how various factors affect the temperature in farm buildings under both summer and winter conditions.

Five different roofing materials were included in this study. They were (1) corrugated aluminum sheet, (2) asbestos-cement shingles, (3) asphalt shingles, (4) galvanized corrugated steel sheet, and (5) wood shingles. Variations in treatment of the materials and in construction of the roofs were as follows: (1) white paint on galvanized corrugated sheet steel, (2) aluminum paint on galvanized corrugated sheet steel, (3) solid wood sheathing under aluminum, and (4) aluminum foil insulation in the roof section with wood shingles.

In the analysis, heat balances were made to determine the temperature each material would attain, and the temperature to which the interior of a building would rise, before reaching equilibrium, with respect to the energy received from the sun and the quantity of heat transmitted to the surrounding air by radiation, conduction, and convection. A study was also made of heat flow outward through the materials under winter conditions. Methods were developed to predict, (a) the quantity of solar energy absorbed and transmitted to the underside of the roof for given conditions, (b) the temperature under a given roof section, and (c) the heat flow outward through roof sections. In the development of these methods, it was assumed that the following were known: (1) the quantity of solar energy incident upon the surface, (2) the absorptivity of the material for solar radiation, (3) the emissivity of the material at ordinary temperatures, (4) the outside temperature, (5) the wind velocity, (6) the thermal capacity of stored products, and (7) the "AU factor" of the understructure.

To check the theoretical hypothesis an experimental study was made. This part of the investigation included four phases of work as follows: (1) the measurement of solar energy absorbed and transmitted to the underside of roof sections by an air duct, (2) the measurement of temperatures under roof sections in insulated compartments

¹ Doctoral thesis number 1062, submitted June 2, 1950.

with zero air change, (3) a field study of temperatures in buildings covered with various roofing materials, and (4) the measurement of heat flow outward through the various roof sections under winter conditions.

In the measurement of solar energy absorbed and transmitted to the underside of roof sections by an air duct, roof sections were placed over a duct through which air was blown. The duct was 6" x 24" x 24'-0" in cross section and was insulated on three sides with 6 inches of corkboard. The duct was mounted on an adjustable frame whereby it could be oriented or elevated so that the roof surface would be normal to the rays of the sun. By measurement of the temperature of the entering and leaving air after thermal equilibrium was established, it was possible to determine the quantity of energy which was transmitted to the underside of the roof section.

In the second phase of the study, the roof sections were placed on a 45-degree slope over well insulated compartments with north and south facings. Temperatures in the middle of the compartments, 2 inches below the inside, on the surface, against the inside and outside surface, and of the outside air were recorded. The quantity of solar energy striking the roof surfaces was measured by a pyrheliometer. Wind velocity was measured by an anemometer. The results obtained in this study checked closely with the theoretical analysis which indicated that roofing material may have considerable effect on the temperature in the building.

The field study included the measurement of temperatures in several typical farm buildings. Buildings included in this portion of the study were, farrowing houses covered with aluminum and asphalt shingles, shade and range shelters covered with five different roofing materials, and a hay mow in a barn covered with aluminum. Although the results were mainly qualitative in nature, they did indicate that considerable variation in temperatures existed in buildings covered with different roofing materials.

The last phase of the study consisted of the determination of U values of roof sections under various winter conditions. The same test house with the insulated compartments which was used in the summer study was used in this study. All compartments were heated with electrical elements to the same thermostatically controlled temperature. By the determination of the energy required to maintain the inside temperature and with the outside temperature known, it was possible to calculate the U values of the roof sections. Again the agreement between the experimental and the theoretical studies was relatively close.

From the results of this study, the following conclusions were drawn:

1. The interior temperature of a building may be accurately calculated by an equation, which was developed in this study, if the characteristics of the roofing materials, the solar heat impinging on the

roof, the outside temperature, the "AU factor" of the understructure, and the quantity of air change are known.

2. The quantity of solar heat absorbed and transmitted to the interior of a building at a given temperature may be readily calculated by an equation which was developed in this study.

3. The temperature of a roofing material and the solar energy absorbed and transmitted by it to the interior of a building are dependent on the characteristics of the material.

4. The main characteristics of a roofing material affecting temperature and heat transmission to the interior are its absorptivity for solar energy, its emissivity at ordinary temperatures, and the conductivity of the material.

5. Roofing materials having approximately equal conductivity coefficients have large differences in temperatures and result in large differences in the interior temperatures if one has a higher absorptivity for solar radiation than the other.

6. Roofing materials having the same absorptivity for solar radiation, but having different emissivities at ordinary temperatures, result in different interior temperatures for a given condition with the material with the highest emissivity having the lowest interior temperatures.

7. A material with high outside emissivity and low inside emissivity is desirable if other things are constant, as this results in a low roofing temperature and the transmission of a small quantity of energy to the interior.

8. The addition of a solid sheathing is more beneficial to materials with high emissivities than to materials with low emissivities.

9. Corrugated aluminum sheet roofing is the most effective of the five roofing materials tested in this study in the prevention of the absorption and the transmission of solar energy to the interior of a building.

10. Solid sheathing is not recommended for corrugated aluminum sheet roofing for the purpose of adding insulation to prevent solar heat from being transmitted to the interior.

11. White paint is the most effective surface treatment in the prevention of the absorption and transmission of solar energy to the interior.

12. Steel painted with white paint is more effective than any other roofing material or surface treatment used in this study in preventing the absorption and transmission of solar energy to the interior.

13. Wind increases the rate of heat loss of the outside surface of the roofing material; hence the equilibrium temperature is lowered and the quantity of heat transmitted to the interior is lowered.

14. The average maximum temperature in buildings covered with the various roofing materials used in this study, ranges from 99.8°F. for insulated wood shingles to 121.9°F. for galvanized corrugated steel for the conditions of this study.

15. The most effective method to prevent the absorption and transmission of solar heat to the interior is to paint the outside surface of the roofing material with white paint and the interior surface with aluminum paint.

THE MECHANISM OF THE FISH THIAMINASE¹

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The physiological function of thiaminase is not known, and there is no evidence to establish whether its action *in vivo* is anabolic or catabolic. Before this question can be answered it is necessary to obtain more knowledge of the chemistry of thiaminase, the reaction mechanism, and the products of its *in vitro* destruction of thiamine.

Our studies were designed to furnish information on these points. Two new activators, *m*-aminobenzoic acid and *m*-nitroaniline, were discovered and were shown to exhibit a strong activating effect in concentrations as low as 5×10^{-4} M. At this concentration activations of 100 to 200 per cent have been measured for undialyzed enzyme preparations, and even higher values were obtained for dialyzed preparations. Activation of undialyzed enzyme by these compounds was not enhanced by manganous ion, but was increased for dialyzed extracts.

In the *m*-nitroaniline-activated reaction the activator concentration decreased during the course of the reaction, and with higher concentrations of activator there were proportionally greater losses. This suggested that the activator was participating in the reaction, and that the pyrimidine moiety of thiamine might be coupling with the amine. This proved to be the case, and the resulting secondary amine, N-(2-methyl-6-aminopyrimidyl-5-methyl)-*m*-nitroaniline, was isolated in a 74.5 per cent yield from the *m*-nitroaniline-activated enzyme reaction. The compound was identical to that produced on warming an alcoholic solution of the corresponding pyrimidine bromide and *m*-nitroaniline in the presence of excess sodium bicarbonate. The dihydrochloride salt of the compound melted, with decomposition, at 211°–212°C. (corrected), and the free base melted at 227.5°–228.5°C. (corrected). Its absorption spectrum exhibited a maximum at 2420–2430 Å. in 0.1 N sodium hydroxide.

Similar attempts to isolate a compound from unactivated enzyme reactions were unsuccessful.

Analysis of the optimum conditions for dialysis of the enzyme indicated that the most satisfactory conditions were those employing a low concentration of enzyme at pH 6.5–7.5 for 6 to 8 hours of dialyzing against distilled water at 5°–10°C. Losses of 50 to 60 per cent of initial activity resulted.

Activation experiments on dialyzed and undialyzed enzyme prepara-

¹ Doctoral thesis number 1007, submitted December 3, 1949.

tions indicated that the manganous ion was intimately associated with thiaminase activity. It was not, however, the sole factor. Another factor, or factors, necessary for optimum activity of the enzyme appeared to be non-dialyzable and non-heat labile and could be replaced by *m*-nitroaniline or Kochsaft, but not by cysteine.

Cysteine exhibited only a slight activating effect on dialyzed preparations. The demonstrated inhibition of thiaminase by sulfhydryl-specific inhibitors, such as iodoacetic acid, phenylmercuric chloride, and iodine, and the reversal of inhibition in the last case by excess cysteine indicate that cysteine might function more as a regulator of the oxidation-reduction state of the enzyme sulfhydryl groups than as an activator such as *m*-nitroaniline.

Cysteine at concentrations higher than 1×10^{-3} M. interfered with the Melnick-Field assay for thiamine. The interference could be avoided by destroying the excess cysteine with iodine prior to addition of the diazotized *p*-aminoacetophenone or by addition of higher concentrations of this reagent.

Attempts to purify thiaminase by fractional precipitation with cold acetone or ammonium sulfate were unsuccessful. The activity was either partly or completely destroyed in every experiment. In view of the recently discovered activators, such as *m*-nitroaniline, this work should now be reinvestigated.

A preparative method was developed for the Melnick-Field dye from thiamine or 4-methyl-5- β -hydroxyethylthiazole methiodide. Both dyes are dense, red-violet, microcrystalline compounds melting above $300^{\circ}\text{C}.$, and their absorption spectra in 0.1 *N* sodium hydroxide have maxima at 268, 310, and 500 Å. for the thiamine derivative and 266, 306, and 490 Å. for the thiazole dye.

In a study of the nature of the Melnick-Field color reaction, the *S*-benzyl derivative of the open form of thiamine was prepared and shown to give no color in this assay procedure.

N-(2-methyl-6-aminopyrimidyl-5-methyl)-taurine was synthesized by the action of the corresponding pyrimidine bromide on taurine in an alcoholic solution containing sufficient sodium bicarbonate to make the mixture slightly alkaline. The compound melts at 211° – $212^{\circ}\text{C}.$ (uncorrected), does not form a hydrochloride, but will form a picrate which melts at 178° – $179^{\circ}\text{C}.$ (uncorrected). The absorption spectrum has two maxima at 235 and 272 Å. in alkaline media and a single maximum at 245 Å in acid media.

SUMMARY

1. Meta-aminobenzoic acid and *m*-nitroaniline were found to be potent activators of both dialyzed and undialyzed thiaminase preparations. Activations of 100–200 per cent were measured for the undialyzed enzyme at activator concentrations of 5×10^{-4} M. Manganous ion at 1×10^{-3} M. concentration enhanced the activating effect for dialyzed enzyme, but not for undialyzed preparations.

2. The product from *m*-nitroaniline activation was isolated in a 74.5 per cent yield and shown to be identical to N-(2-methyl-6-aminopyrimidyl-5-methyl)-*m*-nitroaniline prepared from the corresponding pyrimidine bromide and *m*-nitroaniline. Attempts to isolate a similar pyrimidine derivative from unactivated preparations were unsuccessful.

3. The conditions for optimum dialysis of thiaminase were found to include low enzyme concentration at pH 6.5-7.5 for 6 to 8 hours against distilled water at 5°-10°C.

4. Manganous ion appears to have an integral part in the thiaminase system but is not the only factor. Some other factor, or factors, is necessary which is non-dialyzable and not heat labile and which can be replaced by *m*-nitroaniline or "Kochsaft," but not by cysteine.

5. Thiaminase is inhibited by sulfhydryl-specific inhibitors. Iodine inhibition could be reversed by addition of excess cysteine.

6. The interference of cysteine with the Melnick-Field assay method can be reversed by destroying excess cysteine with iodine prior to the addition of the diazo reagent, or by adding a higher concentration of the diazonium compound.

7. Purification of thiaminase by fractional precipitation with acetone or ammonium sulfate was attempted under various conditions but was unsuccessful.

8. Melnick-Field dyes were prepared from thiamine and from the methiodide of the thiazole moiety and their properties and absorption spectra compared.

9. Two new compounds, the S-benzyl derivative of the open form of thiamine and N-(2-methyl-6-aminopyrimidyl-5-methyl)-taurine, were prepared and their properties and absorption spectra listed.

PROTEOLYTIC AND COAGULATING ENZYMES OF ENTEROCOCCI¹

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Streptococcus liquefaciens, the species which was used in the present study, is a common dairy organism and belongs to the enterococcus group of the streptococci. This organism is known to be resistant to normal pasteurization procedures. In milk it is believed to produce a rennet-like enzyme which brings about rapid coagulation of milk. In addition to this, it causes considerable proteolysis, thereby producing bitterness. The exact nature of the enzyme systems involved in coagulation and proteolysis has not been determined previously.

With a view to gain a better understanding of the role of this organism, the study of its enzyme system as it affects milk proteins was undertaken. Proteolytic activity of the test enzyme was measured by the release of tyrosine and tryptophan from casein substrate at optimum pH 7.4, using intensity of color development with Folin-Ciocalteu's phenol reagent for measurement. Coagulating activity was estimated by noting the time in minutes taken by the test enzyme to coagulate 5 ml. of milk substrate at pH 5.60. The test enzyme used in both these cases was the centrifugally obtained supernatant from several test cultures grown on media of various compositions.

Study of the relationship of nutrition of the organisms to the production of proteolytic and coagulating enzyme activities showed that all nine strains (N, C, B, O, Y, E, 19, 20, and 7) examined produced these two enzyme activities when grown in litmus milk or vitamin-test casein medium. Increase in the concentration of milk solids in litmus milk used as a growth medium resulted in greater production of both types of activities. However, the amounts of the two enzyme activities produced in a buffered peptone broth were considerably less. Addition of either gelatin or casein to this broth resulted in increased production of both enzyme activities. When tryptone, tryptose, proteose-peptone, or peptonized milk was added in the concentration of 1.5 per cent to the litmus milk, decreases in the normal enzyme production occurred.

None of the nine cultures produced detectable amounts of proteolytic or coagulating enzyme activities when grown on Niven and Sherman's simplified amino acid medium, modified to increase its buffering capacity. This points out the adaptive character of the two enzymes.

¹ Doctoral thesis number 1064, submitted June 2, 1950.

Omission of individual vitamins from the vitamin-test casein medium affected both the organism growth and enzyme production. Addition of 2.0 mg vitamin B_{12} per 5 ml. of the vitamin-test casein medium resulted in a three-fold increase in level of both types of activities in the case of culture C but had no effect on the eight other cultures. Such an addition in no case resulted in increased bacterial population. Substitution of equivalent cobalt for B_{12} failed to duplicate the action of the vitamin. Addition of vitamin B_{12} to the enzyme preparation did not influence either activity of the enzyme preparation.

Strains B and C required added thiamine (5 γ per 5 ml.) for growth in the amino acid medium, while the former also required this amount of the vitamin in the vitamin-test casein medium. Additions of thiamine, Tween 80, sodium acetate, ascorbic acid, *p*-aminobenzoic acid, and inositol to the vitamin-test casein medium failed to increase the enzyme production by cultures C, B, and E.

The extracellular proteolytic enzyme has its optimum pH at about 7.4 for digestion of casein and lactalbumin. No such optimum was found for the coagulating activity, the test enzyme showing greater coagulating activity as the pH of the milk substrate decreased to the point where spontaneous coagulation occurred.

Maximum stability of both types of enzyme activity was found to be in the pH range of 6.0 to 8.0. At 61.7°C., both the activities were destroyed in less than 10 minutes over the entire pH range tested (pH 5.0 to 9.0). During 8 days of storage at 2-3° and 30°C., both the proteolytic and coagulating enzymes were stable in the pH range of 5.3 to 9.0, while rapid destruction occurred at pH 4.5 and below.

Addition of sodium oxalate to milk substrate resulted in complete prevention of coagulation by the enzyme. Lowering of pH or addition of CaCl_2 restored the milk coagulating ability of the enzyme. There was a difference in the coagulating action of the test enzyme toward milk substrate heated to different temperatures for a 15-minute period. Milk heated to 70°C. showed maximum susceptibility to the coagulating action of the test enzyme, with decreased coagulating ability when the milk was heated to either higher or lower temperatures. A commercial pepsin preparation tested showed a similar behavior. However, rennet did not act in this manner, its coagulating activity decreasing with increase in heat treatment of the milk. The possibility of a chemical or a physical change in milk proteins or the presence of an enzyme-inhibiting factor in raw milk has been discussed as explanation for this phenomenon.

Different trials using adsorption and salting-out techniques did not result in separation of the proteolytic and the coagulating activities of the test enzyme. Data on heat inactivation, stability of two enzymes and failure to separate them using adsorption and salting-out methods suggest that the proteolytic and coagulating activities probably are two manifestations of a single enzyme.

A peptidase preparation obtained by cell grinding and autolysis was active against glycyl-L-leucine and DL-alanylglycine, the latter being

hydrolyzed far more readily. Two pH optima (8.0 and 5.0) for peptidase action against glycyl-L-leucine were found. Similarly, two pH optima were found for DL-alanylglycine, one at pH 6.9 and the other around pH 8.2; there also was some indication of another optimum around pH 5.0. No detectable activity against either substrate was found at pH 4.0. Hydrolysis of glycyl-L-leucine at pH 8.2 was activated by Mn^{++} , Co^{++} , and Mg^{++} in the declining order stated; hydrolysis of the same substrate at pH 5.0 was activated only by Co^{++} . In the case of DL-alanylglycine at pH 8.3, Mn^{++} , Co^{++} , and Mg^{++} activated the hydrolysis, while only Mg^{++} activated at pH 6.9. Cysteine, Fe^{++} , Cu^{++} , and Ca^{++} had no activating effect for peptidases active on either of the two substrates. The peptidase preparation was not inactivated by 30 minutes, heating at 61.7°C. The results indicate the possibility that several enzymes active only in particular and quite divergent pH ranges are present in the peptidase preparation. There appears to be a rather high specificity in metal activation of the enzyme preparation at different pH levels. Conceivably this could be used to distinguish several dairy organisms from each other.

Thus, both the extracellular proteinase and endocellular peptidases would be expected to be active in cheese, although at a relatively low level due both to low holding temperatures used for ripening and conditions somewhat more acid than optimum for enzyme activity. In addition to this, while the extracellular proteinase would be destroyed in the normal pasteurization of milk, the endocellular peptidases do not appear to be affected by such a treatment. The heat resistance of the latter enzymes, coupled with their activity at about pH 5.0, makes them a potential factor in the ripening process. It is obvious that the action of these enzymes would exert a considerable influence on the flavor and quality of cheese. Possibly, these enzyme preparations could be used to hasten the ripening and to improve quality of cheese.

COUPLED BENDING AND TORSIONAL FREE VIBRATION OF A SWEEP WING¹

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One of the recent trends in the aerodynamic design of high-speed airplanes is toward the use of the swept wing. The determination of the natural frequencies of the free vibrations of such wings is of great importance because the natural frequencies must be used in the determination of the critical flutter speed. The paper presents a method by which the natural frequency of the coupled bending and torsional free vibrations of a swept wing can be approximately determined.

The continuous elastic structure of the actual swept wing is approximated by a composite wing consisting of a finite number of sections. Each section contains a concentrated mass located at the outboard end of the section and an inboard weightless elastic bar of either straight or circular arc planform. The choice as to whether the straight or circular arc bar is used in any section is determined by whichever form more nearly coincides with the locus of elastic centers of the section. The introduction of the circular arc bar also makes it possible to avoid angular discontinuity between sections and at the center of the wing span.

The usual assumptions of thin bar theory are made. Deflections of the vibrating system are assumed small, so that only vertical motion in the direction normal to the plane of the elastic axis and rotation about the elastic axis are considered. Loads other than inertial loads are neglected, and the elements of the sections are assumed to be in harmonic motion.

A set of six equations is developed for a circular arc bar section. Each equation relates a shear, bending moment, slope, deflection, torque, or angle of twist at the inboard end of a section to the shear, bending moment, slope, deflection, torque, and angle of twist at the outboard end. The six relationships are then combined into a single matrix equation for each circular arc bar section.

Then a limiting process is used to derive a matrix equation for a straight bar section from the matrix equation of the circular arc bar section.

One other type of section is introduced to handle such actual concentrated masses as tip tanks, nacelles, and fuselage effects. The matrix equation for this section is obtained from the matrix equation for the

¹ Doctoral thesis number 996, submitted August 20, 1949.

straight bar by setting the length of the bar equal to zero. The swept wing is approximated by a combination of these three types of sections.

After the matrix equations for each section of the wing semi-span have been written, a trial value of the frequency is selected and its value substituted in the section matrices. Then the matrix representing the tip section is premultiplied by the matrix representing the section just inboard of the tip. The product of these two is then premultiplied by the matrix representing the next inboard station. This successive premultiplication is continued until a single matrix equation for the entire wing semi-span is obtained.

The matrix equation for the entire wing semi-span is then reduced to a set of six equations. By this procedure, the boundary conditions at the center of the wing span are related to the boundary conditions at the tip.

At the tip of the wing, the shear, bending moment, and torque are equal to zero. At the midspan, for symmetric free vibrations, the shear, slope, and torque must equal zero. At the midspan, for antisymmetric free vibrations, the bending moment, deflection, and angle of twist must equal zero. If the trial value of the frequency chosen is the frequency of one of the normal modes of symmetric or antisymmetric vibration, the boundary conditions for those modes will be satisfied. If this is not the case, additional values of the frequency must be tried. This trial process is repeated until the natural frequencies are found.

By this method the same basic matrix equations are used to locate both the fundamental and higher natural symmetric and antisymmetric frequencies.

A numerical example of a five-station swept wing is included and the computational techniques are discussed.

COMPARATIVE DEVELOPMENT OF THE EMBRYOS OF INBRED AND HYBRID MAIZE¹

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Previous investigations of heterosis have shown that on the basis of dry weight, hybrid maize embryos may be less than, equal to, or greater than the embryos of parent inbreds. The present investigation was undertaken to explore the possibility that heterosis may be expressed in histological or morphological development of different parts of the maize embryo.

Two inbred lines of dent maize, L317 and B1349, and their reciprocal hybrids were chosen for detailed study after preliminary examinations revealed that these inbreds differed strikingly in histological development. A split-ear pollination technique was employed, whereby inbred and hybrid kernels were grown on the same ears. Embryos of each inbred and each of the reciprocal hybrids were studied 5 days after pollination, and at 5-day intervals thereafter until 40 days after pollination.

Comparisons were made on the basis of visual study of longitudinal and transverse sections of the embryos at 5, 10, and 15 days, and serial transverse sections of embryos from 20 to 40 days after pollination. Four levels were chosen as the chief regions to be compared. These were: (1) the transverse section of the plumule at the level of the apical meristem; (2) the transverse section near the basal portion of the first internode, above the region of seminal root initiation; (3) the transverse section at the level of seminal root initiation; (4) the transverse section of the radicle at the most proximal region at which it is separated from the coleorhiza by a cleft. At levels 1, 2, and 4, visual observations were supported by measurements of transverse sectional areas and by statistical analysis of the data.

The two inbreds chosen for study differ markedly in plumule development with respect to: (1) time of initiation of leaves; (2) activity of marginal meristems of the leaves; (3) transverse sectional areas of plumular organs.

Embryos of the hybrid L317 x B1349, grown on the same ear with the less vigorous parent, L317, surpassed the inbred embryos of the maternal parent in plumular development throughout the entire period of embryonic growth which was studied. The expression of heterosis in this instance consists of: (1) increased leaf initiating activity of the

¹ Doctoral thesis number 1069, submitted June 5, 1950.

apical meristems; (2) increased activity of marginal leaf meristems as judged by lateral expansion of leaves; (3) increased total plumule size as indicated by transverse sectional area. This hybrid, L317 x B1349, does not equal its male parent in plumular development during early embryo growth but is approximately equal to the male parent 40 days after pollination.

Embryos of the hybrid B1349 x L317, grown on the same ear with the more vigorous inbred, B1349, were found to be inferior to the embryos of the maternal parent in plumular development until approximately 35 days after pollination. At 40 days the hybrid B1349 x L317 surpassed the inbred B1349 in transverse sectional area of the coleoptile and the first and fourth leaves, but was otherwise approximately equal to the maternal inbred. This hybrid, B1349 x L317, surpassed its male parent throughout the entire period of plumule growth on the basis of all criteria used in judging comparative development.

The difference between inbreds in rate of leaf initiation which were noted are in agreement with observations of comparative development of these lines after germination. The inbred L317 is a late maturing inbred. The hybrid L317 x B1349 and its reciprocal differ in rate of leaf initiation. Since these two hybrids are genetically identical, the difference might be explained as the result of the maternal influence. The fact that L317 x B1349 equals the more vigorous parent in leaf initiation may indicate that those genes from B1349 which influence this growth process are dominant. The fact that B1349 x L317 exceeds its more vigorous parent in total transverse sectional area might be explained as the result of additive or complementary effect of genes from both parents. An alternative explanation may be that this difference is due to the effect of hybrid endosperm.

The inbreds used in this study are strikingly different in transverse sectional area of the cortex and stele of the first internode. The hybrid embryos, L317 x B1349, grown on the same ear with the less vigorous inbred L317, surpass the inbred embryos in transverse sectional area of cortex and stele of the first internode. The hybrid embryos, B1349 x L317, grown on the same ear with the more vigorous inbred B1349, are equal to the embryos of the maternal inbred in stelar enlargement, but do not become equal in cortical enlargement until after 35 days. Maternal influence in the development of the first internode is evidenced by the fact that the hybrid embryos grown on the more vigorous inbred surpass the embryos of the reciprocal hybrid in transverse sectional area of stele and cortex at all ages.

The inbreds L317 and B1349 are not obviously different in the histological development of the radicle. Differences which do exist consist largely in the relative enlargement of stele and cortex. With respect to stelar enlargement the inbred L317 is superior to B1349 at 30 days but does not maintain this advantage. The inbred B1349 attains a slight superiority in transverse sectional area of the cortex 40 days after pollination. The embryos of the hybrid L317 x B1349 are superior to

those of the inbred L317 in transverse sectional area of the cortex at 30 and 40 days after pollination. However, this hybrid, L317 x B1349, is not superior to its maternal parent in stele at any age. The embryos of the hybrid B1349 x L317 are superior to those of the inbred B1349 in transverse sectional area of both stele and cortex at 30 days. By 35 days after pollination this hybrid, B1349 x L317, has lost its superiority in transverse sectional area of cortex, but exceeds its maternal parent in transverse sectional area of stele at all ages.

The above comparisons indicate that L317 contributes favorable genes for the growth of stele, while B1349 contributes favorable genes for growth of cortex. Therefore, heterosis in the radicle is the result of the combined effect of favorable genes that are contributed by both parents, and which influence histogenesis.

In seminal root development, the outstanding difference between the inbreds is the time of root initiation. Seminal root development in B1349 is more advanced than in L317, whereas, both hybrids approach the more advanced parent. This situation agrees with respective development in the plumule. The genes which influence early initiation of seminal roots appear to be dominant. A maternal effect is indicated by the fact that seminal root development in the hybrid embryos, grown on the same ear with the more advanced inbred embryos, exceeds seminal root development in hybrid embryos, grown on the same ear with the less advanced inbred embryos.

TOXICITY OF INGESTED
GAMMA-1,2,3,4,5,6 BENZENE HEXACHLORIDE (LINDANE)
TO ALBINO RATS¹

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The extensive use of synthetic organic insecticides has created a potential hazard to man and domestic animals. The possibility of acute or chronic toxicity reactions in mammals exposed to these insecticides warrants thorough investigation before such chemicals are released for general use.

One of the synthetic organic insecticides receiving considerable attention is gamma-1,2,3,4,5,6 benzene hexachloride (lindane). The present work was undertaken in order to determine the effect on albino rats of ingesting small amounts of this insecticide over prolonged periods. The experimental diet consisted of stock rat ration containing 500 parts per million of gamma benzene hexachloride.

It was found that weanling or older rats of the Wistar-Ames strain tolerated the experimental diet for nine months or more without evident external symptoms of toxicity at the time of sacrifice. However, if the feeding of the experimental diet to younger rats was continued until natural death, experimental female rats tended to have a shorter life span than control females. Experimental male rats of this particular strain did not exhibit this tendency toward reduced longevity.

When animals were first placed on the diet containing gamma benzene hexachloride, a decline in growth rate was evident for 2 to 4 days. This decline was more pronounced in female than in male rats. The addition of inositol to the diet did not prevent the initial drop in body weight in experimental animals. It was noted that the beginning weight loss was closely correlated with a reduced food intake.

Data are presented to show that young rats were able to differentiate treated from untreated food. When given a choice between treated and untreated food over an extended period, young rats consumed a progressively greater percentage of non-treated food. Male rats were more sensitive to the presence of the contaminant in the food than were female rats.

Although the reproductive ability of rats was not appreciably altered when gamma benzene hexachloride was included in the diet, marked changes in the ability of productive females to care for their young were evident. Three- to 6-month-old females maintained on the

¹ Doctoral thesis number 1047, submitted May 19, 1950.

experimental diet for an average of three months reared approximately 16 per cent of their young to weaning. This was in contrast to corresponding control females who reared approximately 73 per cent of their young to weaning. Generally, the longer the female rats were maintained on a diet containing 500 parts per million of gamma benzene hexachloride, the less their inclination to care for young. Experimental females apparently did not develop any tolerance for the toxicant inasmuch as the rearing performance did not improve with time. However, the detrimental effects, seemingly attributable to ingestion of the gamma isomer, disappeared when experimental females were placed on normal food at the time of subsequent mating.

The continual ingestion of the experimental diet did not produce any significant changes in either the erythrocyte or leucocyte counts in albino rats.

Experimental rats, after ingesting treated food for 2 months or more, showed an increase in the relative weight of the liver. Likewise, an increase in the relative weight of the kidneys was evident in all age groups of experimental males and in females maintained on the experimental diet for 6 months or more. Although there was a trend toward an increase in the kidney weights of females that had been on the experimental diet for 2 months, the difference at this time between the normal and experimental weights of this organ was not significant.

The relative increase in the liver-body weight ratio was similar in experimental males and females; however, the relative increase of the kidney weight of experimental males was greater than in experimental females.

No significant changes were found in weights of spleen, adrenals, and testes in rats ingesting experimental food over prolonged periods.

When examined histologically, liver damage was evident in rats maintained on the experimental diet. However, only rarely was this damage great enough to be considered irreparable. Evidence of a chronic toxicity state in the liver was indicated by albuminous and fatty degeneration. Generally, the kidneys appeared quite normal when examined microscopically although some hyaline degeneration was evident.

Using a bioassay to detect the presence of gamma benzene hexachloride in tissues, data indicated that rats were able to eliminate this toxicant from their tissues in a very short time. After 12 days on normal food, blood, muscle, liver, and even kidney and adipose tissues from females that had been on the experimental diet for a year contained only traces of the toxicant.

GLYCEROL FERMENTATION OF STARCH¹

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Most of the fermentation procedures for glycerol that have been described in the literature use sugar as the raw material, but it would be desirable from the standpoint of initial cost to use starch. There are very few references to the use of starch for this fermentation although it has been suggested in a few instances. An important difficulty in the usual sulfite process is the recovery of the glycerol from the fermented material. The high concentration of soluble salts resulting from the use of large amounts of sodium sulfite or bisulfite is the principal source of the difficulty. The purpose of the investigation on which this thesis is based was to determine the best conditions for a glycerol fermentation process using starch as the fermentation substrate. It was hoped that a process could be developed which might have practical application for commercial glycerol production. To this end most of the work was done with the use of those sulfites which would not increase the concentration of soluble salts enough to make the glycerol recovery too difficult.

Acid-hydrolyzed starch was found to provide a suitable substrate for glycerol fermentations. In all cases tried the starch medium gave practically as good yields as those reported for sugar medium. Sulfuric acid concentrations of 0.1 normal gave 96 per cent conversions to reducing sugars of 10 per cent starch mashes when heated for 2 hours at steam pressures of 25 pounds per square inch. From these mashes yields of 23 per cent glycerol, based on the glucose equivalent of the starch, were obtained. Magnesium sulfite hexahydrate was used in amounts equal to the weight of the sugar, and inoculation was made with 15 ml. of a suspension of yeast cakes for each 300 ml. of mash. The yeast suspension contained one yeast cake in each 45 ml. of a 5 per cent glucose-0.5 per cent corn steep liquor medium and was incubated at 30°C. for 6 hours before it was used for inoculation. The fermentations themselves were also carried out at 30°C. for 65 hours.

Sulfur dioxide in concentrations from 0.5 to 1.5 g. per 100 g. of water gave 67 to 80 per cent conversions of the starch to reducing sugar when heated 2 hours at 30 pounds steam pressure. The glycerol yields from magnesium sulfite fermentations of these mashes ranged from 14 per cent for the lower concentration of sulfur dioxide to 8 per cent for the higher concentration.

¹ Doctoral thesis number 989, submitted August 3, 1949.

The addition of nutrients to glycerol fermentations of acid-hydrolyzed starch was unnecessary if the large inocula described above were used. The fermentations were brought about apparently by the enzymes associated with the inocula, and there was very little proliferation of the yeast.

Magnesium, calcium, and ammonium sulfites gave yields of 23, 6, and 2 per cent glycerol, respectively. The addition of 30 g. of magnesium sulfate heptahydrate to calcium sulfite fermentations containing 30 g. of calcium sulfite dihydrate increased the glycerol yield to 10 per cent. Magnesium sulfite and yeast can be used over for successive fermentations if care is taken to maintain the activity of the yeast. The use of mixtures of magnesium, calcium, and ammonium sulfites gave poorer yields of glycerol than the use of magnesium sulfite alone. For example, a mixture of 20 g. of magnesium sulfite hexahydrate and 10 g. of calcium sulfite dihydrate gave a glycerol yield of 19 per cent, and a mixture of 30 g. of the magnesium salt, 20 g. of the calcium salt, and 10 g. of ammonium sulfite monohydrate gave only 3 per cent. Ammonium sulfite actually inhibited the fermentation so that its use in mixtures prevented the other sulfites from giving their normal yields of glycerol. Addition of 15 g. of sodium sulfite heptahydrate to a magnesium sulfite fermentation containing 15 g. of magnesium sulfite hexahydrate increased the glycerol yield to 26 per cent.

The pH of the fermentations media influenced the yields of glycerol. For magnesium sulfite the optimum value lay between 6.0 and 6.5, and for the calcium sulfite it was about 5.5. When ammonium sulfite was used, the variation of glycerol yields with pH was not great for pH values between 4.5 and 6.5. The addition of sulfur dioxide to control the pH during the fermentations did not appear to be desirable.

The percentage yields of glycerol decreased from 23 when the initial concentration of starch was 5 per cent to 17 when a 20 per cent acid-hydrolyzed starch mash was used. A similar effect was observed in series of fermentations conducted at 37°C. as well as those at 30°C. It was found that the yields of glycerol obtained at 37°C. were about 0.5 per cent higher than those at 30°C. for the same starch concentrations. Stirring was advantageous where the less soluble salts were used in the fermentations. Delaying the addition of the sulfite for 2 hours after inoculation of the fermentations decreased the glycerol yield to 20 per cent. The addition of sulfite to the yeast suspension to acclimatize it to the sulfite before inoculation did not improve the glycerol yields.

Glycerol can be produced from acid-hydrolyzed starch by *Bacillus subtilis*, Ford's strain. The maximum yield was about 22 per cent, and no aldehyde-fixing agent was required. The fermentations were inhibited increasingly by increasing additions of sulfite until the yield of glycerol had decreased to 3 per cent when 8 per cent of magnesium sulfite hexahydrate was added.

The yeast *Zygosaccharomyces acidifaciens* fermented acid-hydrolyzed starch without an aldehyde-fixing agent to give about 8 per cent

glycerol. It was found that the addition of 10 per cent magnesium sulfite hexahydrate to the fermentation increased the glycerol yield to 23 per cent. The acetaldehyde content of the fermented material increased from a negligible value in the fermentations without sulfite to a value almost equivalent to the glycerol in the fermentations where sulfite was used.

DETECTION OF NECROTIC RING SPOT VIRUS AND ITS OCCURRENCE AND SPREAD IN SOUR CHERRY NURSERIES¹

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The problems involved in the production of virus-free sour cherry trees in nurseries have been meagerly investigated in the past. Since viruses are not usually transmitted through seed, the basic assumption was that propagation of cherry trees with buds from known virus-free scion trees would solve the major problems concerned. Because virus-free budwood of several varieties was available at the outset of these investigations, it seemed that the important information needed was that which would give assurance that virus-free trees grown under isolated conditions would not become contaminated with virus. Such information should be concerned with the occurrence of virus in *Prunus* cultivated in or growing near the nurseries and with the presence or absence of virus spread in the cherry nursery. The report by Cation (6) that viruses may be transmitted through *P. Mahaleb* seed and circumstantial evidence that a portion of *P. Mahaleb* cherry rootstocks are virus infected made it imperative that the status of the *P. Mahaleb* seed trees and *P. Mahaleb* seedlings be clarified.

Investigations for acquiring quantitative data were impeded by lack of a reliable index plant which could be made available in large numbers. Very early in these investigations *P. tomentosa* gave promise of being such a plant, so that considerable information was accumulated to determine its value.

The reliability of *P. tomentosa* as an index plant for necrotic ring spot virus was tested by comparing it to Elberta peach in the field and Montmorency cherry in the greenhouse.

In the field *P. tomentosa* detected virus in more of the trees indexed than did Elberta peach. Ten of 199 *Prunus Mahaleb* L. orchard trees were found infected by indexing on *P. tomentosa*, while only seven were found with Elberta peach seedlings. All of 111 Early Richmond cherry nursery trees were found to be infected by indexing on *P. tomentosa*; only forty-one were found with peach. None of twenty Waneta plum and twenty *P. Besseyi* were found to be infected with either the *P. tomentosa* or the peach seedling index.

In the greenhouse comparison, *P. tomentosa* seedlings and Montmorency cherry trees were inoculated with nineteen strains of necrotic ring spot virus, three strains of prune dwarf virus and one strain of

¹ Doctoral thesis number 1060, submitted June 1, 1950.

peach rosette mosaic virus. Both index plants reacted to all nineteen strains of necrotic ring spot and three strains of prune dwarf virus. *P. tomentosa* reacted to peach rosette mosaic virus while Montmorency cherry did not. Symptoms induced by the virus on *P. tomentosa* consisted of chlorotic mottling with or without necrotic rings on the leaves of new growth, accompanied in some cases with necrosis of terminal growth. Virus strains could not be differentiated by symptom expression. In these experiments *P. tomentosa* was more reliable than Elberta peach in the field and as reliable as Montmorency cherry in the greenhouse as an index plant for necrotic ring spot virus.

Samples of seventeen varieties of plums, five varieties of ornamental *Prunus*, one variety of apricot and six varieties of cherries cultivated in southwest Iowa nurseries were indexed for necrotic ring spot virus. Of these thirty-two species and varieties, twenty-seven were found to contain virus. Infection ranged from 0 to 70 per cent in twenty-six of the species and varieties. In only one, Early Richmond, was virus present in 100 per cent of the trees. A total of 496 individual trees were indexed in this experiment. Average infection for all samples was found to be 37 per cent (confidence interval, 32 to 42 per cent, $P = 0.95$).

Two samples of Wild Black Cherry and two samples of Wild Plum were indexed. Infection in the Wild Black Cherry samples was found to be 50 and 55 per cent (confidence intervals, 17 to 83 per cent and 36 to 70 per cent). Infection in the samples of Wild Plum was found to be 14 and 55 per cent (confidence intervals, 3 to 37 per cent and 30 to 79 per cent).

These data indicate that most of the *Prunus* species and varieties cultivated in southwest Iowa nurseries and two of the wild species growing near the nurseries are infected with necrotic ring spot. Isolation of virus-free sour cherry nursery stock from these infected *Prunus* is necessary. The possibility of Wild Plum rootstocks as sources of virus contamination for cultivated plum is also indicated.

One hundred and sixty-one *P. Mahaleb* L. trees composing a seed orchard near Gustine, California, and twenty-five samples of budwood from a *P. Mahaleb* orchard near Zillah, Washington, were indexed. Seven per cent of the California orchard trees and 85 per cent of the samples from the Washington orchard were found infected. Three samples from fields of *P. Mahaleb* seedlings were indexed; these samples were found to contain 14, 35, and 18 per cent infection (confidence intervals, 6 to 27, 15 to 59, and 12 to 24 per cent, respectively).

The data definitely establish that virus is present in *P. Mahaleb* orchard trees and that some of the seedlings being shipped into southwest Iowa nurseries are infected with necrotic ring spot virus and constitute one means of introducing this virus into sour cherry nursery stock in that area. The possibility of spread of virus in the *P. Mahaleb* seedling bed is suggested.

In an attempt to determine the rate of spread of necrotic ring spot in sour cherry nursery stock in southwest Iowa, random samples of trees

in three fields were indexed at two different dates. Two of these fields were isolated by a distance of at least one-fourth mile from other *Prunus*; the other was surrounded by sour and sweet cherries known to contain 32 per cent ring spot (confidence interval, 14 to 48 per cent).

According to the chi-square test, in the unisolated field there was a significant increase in percentage of infected trees during the time interval between indexing dates. In this field percentage of infected trees increased from 9 to 37 per cent during a 46-day interval. In the isolated fields the percentage of infected trees increased from 2 to 8 per cent in 46 days in one, and from 18 to 20 per cent in 1 year in the other.

It seems that spread of necrotic ring spot virus may occur in sour cherry nursery stock propagated in southwest Iowa and that isolation of sour cherry fields by one-fourth mile from other *Prunus* may help ensure their continued freedom from contamination by necrotic ring spot virus.

FUNDAMENTAL ASPECTS OF DEPRECIATION THEORY ¹

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Increased interest in depreciation has arisen because of the high income tax rates and excess profits taxes during and following World War II. This is evidenced by (1) the controversies between tax agencies and companies concerning the exact amounts of depreciation to be allowed and (2) the concern of both government and business over the indirect effects of depreciation policies upon the modernization of industrial properties. Prior to 1934 when T.D. 4422 was issued depreciation was of more interest to those few individuals concerned with public utility rate making than to the average businessman. Depreciation is now of concern to all people who own properties which have a net taxable income. An understanding of the general principles upon which estimates of depreciation are based will assure the taxpayer the payment of a minimum income tax in so far as cost-depreciation affects it. It also will provide a better estimate of the financial position of his business with regard to the investment in long-lived properties. Recent literature on depreciation has contained so many assertions based upon implicit assumptions and vague generalities that this study of the underlying principles of depreciation has been undertaken. The principal objective of the study has been to integrate and explain the ideas on depreciation which already exist in diverse places and whenever possible to compare alternative proposals for estimating depreciation. It is an effort to clarify the basic issues which underly much of the present controversy about depreciation.

A survey of the historical development of the idea of allocating the cost or value of a property over the life of the property reveals that when the word depreciation was introduced it was used in either the sense of cost or of value and was ambiguous from the beginning. Since that time depreciation has been used to connote the physical condition of a property without regard to the allocation of either cost or value. Much confusion in discussions of depreciation has arisen because the exact meaning of the word depreciation was never specified in the discussion. Of prime importance in any discussion of depreciation in either of the above specific meanings is the meticulous qualification of the word depreciation throughout the discussion.

A clear statement of the objectives of any depreciation policy will do much to facilitate a choice of a method whereby these objectives can

¹ Doctoral thesis number 1049, submitted May 23, 1950.

be attained. One of the principal objectives of nearly every depreciation policy is the allocation of the cost of the property over the life of the property. Depreciation in the sense of a decrease in value has little significance when applied to commercial problems.

Two other recognized objectives are (1) the allocation of the cost in proportion to the service rendered and (2) the allocation of the cost of each unit of property over the life of the unit. The first objective generally implies that the services rendered by a property unit are homogeneous, and are allotted equal increments of cost. These two objectives are incompatible when applied to group properties. The average life method allocates equal cost to each unit of service. The unit summation method allocates the cost of each unit of property within the group over the life of that unit. However, neither method satisfies both objectives. When either method is applied to a stabilized continuous property, the same annual allotment will be obtained. The difference between the average life method and the unit summation method of allocating the cost of a stabilized continuous group lies in the size of the reserve. The unit summation method, when applied until a non-growing group stabilizes, will yield a reserve of 50 per cent of the depreciable cost regardless of the property group. The average life method generally will yield a reserve of from 30 to 50 per cent of the depreciable cost depending upon the mortality characteristics of the property.

Interest on the investment, the cost of replacement, and the time of replacement have been included in depreciation theory. The use of an interest method should generally be omitted because the use of a compound interest formula intensifies in the later life of the property errors inherent in the estimation of the life, salvage value, and interest rate. The use of the replacement cost of a property as the basis of allocation to assure the investor a protection of his investment is not recommended because replacement cost is not necessarily an index of the stability of the investment. The word replacement is related to depreciation theory only because the date of replacement, dictated by replacement theory, is the date of retirement of the property which is replaced.

The adjustment of the cost-depreciation allocations occasioned by a revision of the forecasts of probable life and salvage value of the property have been unduly neglected. It has been shown that the pattern of the allocations can be as greatly influenced by the method of adjustment of the reserve for cost-depreciation and the annual allotment as by the method of allocation. For example, the straight-line method will yield an entirely different distribution of allocations over the life of the property when the changes in forecasts are either ignored, adjusted by spreading the unallocated cost over the remaining life, or adjusted by an entry in the surplus account and basing the annual allocation on the latest estimates. Since it is not possible to forecast either the life or salvage value of a property accurately when it is purchased,

it is necessary to change the forecast when conditions warrant. Cost-depreciation estimates should be adjusted accordingly if the usual objectives are to be attained.

The application of cost-depreciation methods to income tax computation has aroused much controversy. One of the more controversial aspects of these estimates is set forth in T.D. 4422. In this decision the former policy of allowing business wide latitude in fixing its rates was reversed, requiring businesses to submit proof of cost-depreciation rates if these rates are questioned. On the basis of the total taxes received for periods of ten years or more there appears little reason why the Bureau of Internal Revenue should continue to insist upon this regulation of rates provided the three existing restrictions are maintained: viz., (1) no more than the cost of the property can be allocated (deducted), (2) the taxpayer must maintain a consistent cost-depreciation policy, and (3) cost-depreciation must be taken when it occurs (is allowable). With these restrictions, a business which has a net taxable income each year will pay a minimum of taxes when the life of the property is correctly estimated. Errors in estimating the rates of depreciation will result in more, not less, taxes and thus become a concern of business, not of the Bureau of Internal Revenue. The effect of incorrect forecasts will probably be less influential on the taxes when continuous group property methods are used than when either the individual unit methods or the original group property method is used.

The integration of the theories and methods related to the estimation of depreciation is a large undertaking of which this study is only a small part. This study has attempted to explain and criticize rather than advocate a new approach. It is hoped that further examination of these fundamental ideas upon which the concept of depreciation is based will lead to better estimates of depreciation and less controversy over the subject of depreciation.

FRACTIONATION OF THE EGG WHITE PROTEINS IN MEDIA OF LOW DIELECTRIC CONSTANT AND LOW IONIC STRENGTH¹

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That egg white is a protein system of biological importance is well recognized. The availability of egg white protein fractions should promote research into their physiological role in embryonic development as well as various technological aspects of eggs and egg products. One of the primary limitations to the study of the physical chemistry of the egg white proteins has been the lack of a satisfactory means of preparation in pure form. The successes of Cohn and co-workers at the Harvard Medical School in fractionating the plasma proteins in media of low dielectric constant and low ionic strength prompted this investigation into the possibility of applying their procedures to the egg white system.

A preliminary investigation of the electrophoretic analysis of egg white was made so that composition data would be available and yields of the various proteins could be determined during the fractionation experiments. The electrophoretic mobilities of the egg white proteins were needed to aid in identification of the various fractions obtained.

In previous electrophoretic investigations of egg white, ovomucin was removed prior to the experiment because of its insolubility under the conditions employed. Suitable conditions were discovered so that ovomucin remained in solution during electrophoretic runs. Ovomucin remains in solution when the pH is maintained between 6.5-8.0 and the ionic strength at 0.15 or above.

Optimal electrophoretic resolution was obtained at pH 7.7-7.8, $r/2 = 0.20$. Thirty-one electrophoretic runs were carried out under these conditions to determine the mean relative composition and mobilities of the egg white proteins. The results were treated statistically and the source of the errors discussed. The average protein composition of whole normal egg white was found to be: ovalbumin, 64.9 per cent; ovomucoid, 9.2 per cent; globulin, 8.7 per cent; conalbumin, 13.8 per cent; lysozyme, 3.4 per cent; ovomucin, 1.1 per cent (by isolation). Under the reference conditions chosen lysozyme migrates in the opposite direction from the rest of the proteins, and the data regarding its mobility and concentration are of doubtful value.

Several factors were considered which might affect the electrophoretic analysis of egg white. In the normal patterns, discussed above, in which ovomucin was included it could not be identified. Enrichment

¹ Doctoral thesis number 992, submitted August 15, 1949.

of the egg white with ovomucin did not alter significantly the electrophoretic patterns.

Changes in pH and ionic strength effected only a slight modification of the electrophoretic composition. The electrophoretic mobilities of all constituents were strongly dependent upon both of these factors. The mobilities of all components increased with decreasing ionic strength at pH 7.8.

Neither the age of the eggs nor genetic differences in the chicken influenced the electrophoretic analyses under reference conditions. Studies under other conditions were not possible without removal of ovomucin or a decrease in resolution.

During the electrophoretic investigations there were several indications that protein-protein interactions might occur under the reference conditions. Appropriate mixtures of lysozyme with conalbumin, ovalbumin, or ovomucoid were studied electrophoretically, but no evidence for interaction was obtained.

It appeared that several of the fractions of egg white might be best identified by determining the amount of hexose which they contained. Since egg white contained 0.45 per cent free hexose, it was necessary to remove this sugar before the bound carbohydrate could be determined. Experiments were carried out to determine whether free hexose could be removed by dialysis. The results indicated that this free carbohydrate could be removed for analytical purposes on small samples but that it was not feasible on a large scale with the usual laboratory apparatus.

A comprehensive fractionation scheme was developed in which all of the major protein components of egg white were obtained in varying degrees of purity. Separation of the various fractions was effected by control of five variables: pH, ionic strength, ethanol concentration, protein concentration, and temperature. All fractions were removed at temperatures within 1-2° of the freezing points of the solutions.

Ovomucin, the first fraction, was removed by super-centrifugation (45,000 x G) without any other treatment of the egg white. This procedure probably results in an ovomucin preparation with less impurities than any previously described.

Conalbumin and globulin were removed together as the second fraction by adjusting the pH to 6.3, the ionic strength to 0.05, the protein concentration to 8-10 grams of N/liter, and adding ethyl alcohol to a mole fraction of 0.07. Only about 50 per cent of the total globulin was removed under these conditions. The remainder was not precipitated until the alcohol concentration was increased. This suggested the possibility that the latter might be a pseudo-globulin. The lysozyme was precipitated with this fraction and could be crystallized from it by adjusting the pH to 9.5 and adding NaCl to a final concentration of 5 per cent. The globulin could be partially removed from the conalbumin by dialyzing against water to reduce the ionic strength to approximately

zero. The yellow color of egg white, presumably riboflavin, was precipitated with the conalbumin-globulin fraction, and when the globulin was removed as described, the colored constituent was found with the conalbumin fraction. Further purification of the conalbumin indicated that the riboflavin was not bound to the characteristic conalbumin component, but rather to a protein which was insoluble in water but soluble in 5 per cent salt solutions. It was proposed that this component binding the riboflavin might be a globulin, or a minor portion of the conalbumin constituent, with different solubility characteristics than usually attributed to albumins.

Following the removal of the conalbumin-globulin fraction, the ovalbumin fraction was removed by lowering the pH to 4.6, the ionic strength to 0.01–0.03, and increasing the mole fraction of ethanol to 0.11. The solubility of the ovalbumin was strongly dependent upon the tem-

TABLE 1
DISTRIBUTION OF THE EGG WHITE PROTEINS INTO FRACTIONS.
FRACTIONATION EXPERIMENT F-13.

Fraction	Ovalbumin		Ovomucoid		Globulin		Conalbumin		Lysozyme	
	(1)*	(2)†	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
Egg White.....	64.9		9.2		8.7		13.8		3.4	
II-1.....	13.4	1.2	9.5	6.1	61.0	41.5	2.9	1.2	12.4	21.7
II-2-B- α -1.....	14.7	1.2	2.1	1.2	17.9	11.0	41.0	16.2	19.0	20.0
II-2-B- α -2.....	19.4	2.8			5.4	5.8	75.8	52.0		
III-2-A-2.....	93.7	67.2	2.1	10.4	2.9	15.5	1.5	5.0		
IV.....	11.1	0.5	88.9	29.3						
Total Yield.....		72.9		47.0		73.8		74.4		41.7

* (1) Percent of constituent in the fraction.

† (2) Percent of constituent in the fraction of that originally present in the egg white.

perature under the conditions of this removal and considerable difficulty was encountered in obtaining complete removal. The temperature coefficient of solubility of all the egg white proteins was very high in ethanol-water solutions.

The ovomucoid was recovered from the supernatant of the ovalbumin precipitation. The solution was concentrated by pervaporation, alcohol, and salts removed by dialysis, and dry ovomucoid preparations obtained by lyophilization.

The results obtained in a typical fractionation experiment are shown in Table 1. The purity of all fractions can be readily improved by purification procedures similar to those employed for the initial removal. The yields of the various proteins depend primarily upon the ability of the operator in holding mechanical losses at a minimum. Yields might be increased by inclusion of all washings in subsequent steps.

HISTOCYTOLOGICAL STUDIES ON NORMAL BOVINE LIVERS AND ON BOVINE LIVERS EXHIBITING A FOCAL HEPATITIS AND TELANGIECTASIS¹

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A knowledge of the normal histological and cytological picture of an organ, with its normal variations, is an essential prerequisite for the recognition of any pathological deviation from the normal. With this in mind, cytological and histochemical observations were made with the hope of establishing a definite microscopic picture of the normal bovine liver under the conditions of this investigation. Histochemical and cytological studies were also concurrently made of so-called sawdust² and telangiectatic bovine livers.

The writer (8) has previously indicated that the meat-packing industry has sustained a heavy monetary loss for years due to the condemnation, under federal inspection, of bovine livers affected with telangiectasis and the so-called sawdust condition. Since the bovine liver has materially increased in monetary value and nutritional significance in recent years, it seemed advisable to first establish a definite normal microscopic picture of this organ. The establishment of such a picture may in turn facilitate further research and investigation pertaining to this organ.

During the course of this investigation, approximately 5,000 sections, taken from sixty-nine bovine livers, were examined both macroscopically and microscopically. A complete history of the animals from which the material was taken was available and the disposition of the carcass, the diet, type of pasture, the age, and time of last feeding and watering were all known. The acid and alkaline phosphatase activity, the cell organelles, glycogen deposition, and fat deposition were routinely studied. It was deemed advisable to study in detail the cell organelles since numerous investigators have demonstrated that mitochondrial and Golgi changes are often the most sensitive and earliest indications microscopically of cellular changes and damage. The phosphatases were also studied since alkaline and acid phosphatase have been observed to vary under various physiological and pathological conditions.

The livers used in this study were obtained from animals slaughtered at the plant of the Bookey Packing Company, located at Des Moines,

¹ Doctoral thesis number 984, submitted July 15, 1949.

² Term applied by the Meat Inspection Service of the U. S. Department of Agriculture to bovine livers exhibiting a focal hepatitis.

Iowa. Both steers and heifers, varying in age from 1½ to 3 years were slaughtered. All of the animals were in prime condition, many having been shown at the Iowa State Fair. They included the Hereford, the Aberdeen Angus, and the Shorthorn breeds. After the animals were killed by a blow on the head and bled by way of the jugular vein, the liver specimens were immediately placed in the various fixatives employed and returned to the laboratory at Iowa State College for embedding, sectioning, and mounting.

For a general fixative, a 10 per cent solution of neutral formalin was used. For mitochondrial studies, Regaud's technique as modified by Deane (3) was followed. Duplicate samples were also fixed in Bensley's (1) acetic osmic bichromate solution. For the demonstration of the Golgi apparatus, Nasonov's modification of Champy's fluid was used with postossification as suggested by Kolatchev and described by Bowen (2). For the demonstration of glycogen, the recommended technique of Bensley (1) was followed. For the demonstration of fat, the tissue was fixed in a 10 per cent neutral formalin for 48 hours and embedded in gelatin as described by Zwemer and modified by Deane (3). The methods of Gomori as modified by Deane and Dempsey (4) were followed for the histochemical demonstration of phosphatases.

The following points may be emphasized from the results of this investigation:

1. In the normal liver and in the liver exhibiting a focal hepatitis and telangiectasis, the alkaline and acid phosphatase enzymatic activity was more intense in the portal canal area and in the peripheral cells of the lobule than in the central portion of the hepatic lobule.

2. Alkaline and acid phosphatase was observed to be present in the cytoplasm and nuclei of the hepatic cells, the bile canaliculi, the lining cells of the sinusoids and blood vessels, the cells of the bile ducts, and in variable amounts in the leucocytes and lymphocytes.

3. Less phosphatase activity occurred in the normal bovine liver, the telangiectatic liver and the liver exhibiting a focal hepatitis, at pH 4.7 to 5.0 than in the alkaline range.

4. In the telangiectatic bovine liver, alkaline glycerophosphatase (pH 9.5 to 9.0) and acid glycerophosphatase (pH 4.7 to 5.0) were demonstrated in similar sites and exhibited similar activity to that observed in the normal bovine liver.

5. The bovine liver exhibiting a focal hepatitis revealed a very intense concentration of both acid and alkaline phosphatase within and about the foci of necrosis.

6. In the livers exhibiting a focal hepatitis, the hepatic parenchyma exclusive of the necrotic areas revealed a normal distribution of alkaline and acid phosphatase.

7. The observations as to the distribution of alkaline and acid phosphatase in those livers exhibiting a focal hepatitis and telangiectasis, would not seem to reveal an enzymatic picture characteristic of malignant tumors as described for other species.

8. A characteristic mitochondrial pattern was noted in the hepatic cells located in the peripheral and central portions of the normal hepatic lobule.

9. In the normal bovine liver, the mitochondria were peripherally located in the hepatic cells of the central lobular area in contrast to a diffuse distribution within the hepatic cells of the peripheral portion of the lobule.

10. The cytoplasm of the hepatic cells in and contiguous to the necrotic foci seen in livers exhibiting a focal hepatitis appeared very dark due to the clumping of the mitochondria into large osmiophilic masses.

11. The telangiectatic liver revealed throughout a mitochondrial pattern comparable to that observed for the normal hepatic cell.

12. The Golgi substance in the apparently normal bovine liver was fused into the form of tight networks both juxtannuclearly and peripherally located.

13. The Golgi material of the hepatic cells within the telangiectatic area was arranged in a continuous network which imparted a fenestrated appearance to the cells.

14. The Golgi substance in the cells contiguous to and within the necrotic foci of the "sawdust" liver was fused into a tight, strongly osmiophilic mass which at times completely obliterated all other cytoplasmic detail.

15. The observation that both the mitochondria and the Golgi material are clumped in the hepatic cells in, and contiguous to, the necrotic foci seen in the "sawdust" liver would lend support to the belief that normal metabolic processes have been altered in the bovine liver exhibiting a focal hepatitis.

16. The mitochondrial and Golgi pattern in the telangiectatic liver remains within the realm of normal physiological variation.

17. The discrepancy in our present literature regarding glycogen deposition in the hepatic lobule is no doubt due to the lack of conformity to a definite time interval between feeding time and killing time.

18. Various lipoidal substances were observed in all the hepatic cells of the normal bovine hepatic lobule.

19. The neutral fats appeared centrally, whereas the fatty acids and other fatlike substances which appeared in lesser amounts were seen peripherally in the normal hepatic lobule.

20. One must at all times be cognizant of the variability in histochemical and cytological structure of the hepatic cell due to its functional activity.

21. No apparent increase or decrease in connective tissue fibers or elastic tissue fibers was noted in those livers exhibiting focal hepatitis and telangiectasis.

22. Both telangiectatic areas and necrotic foci were noted macroscopically in the same liver and microscopically in the same section.

23. Many livers, designated as "normal" grossly, exhibited a focal hepatitis and telangiectasis microscopically.

24. Since the phosphatase activity, the mitochondrial pattern, the Golgi pattern, the glycogen deposition and the fat deposition within the livers exhibiting telangiectasis resembled that observed histologically in the normal bovine liver, it is concluded that normal metabolic processes are unimpaired in this condition.

25. Since the phosphatase activity, the mitochondrial pattern, and the Golgi pattern, in those livers exhibiting a focal hepatitis, presented a different histological picture than that observed in the normal bovine liver, it is concluded that normal metabolic processes are altered in bovine focal hepatitis.

26. All carcasses of which the livers showed the lesions discussed were passed for human food; no other concurrent lesions were evident grossly.

In view of the microscopic findings of this investigation, it would next seem advisable to consider this problem from a nutritional basis. Since no proof to date has been revealed that telangiectasis and the sawdust condition are of an infectious origin, and since the recognition of purely nutritional factors as important agents causing hepatic disturbances is well confirmed, the attempt to prevent telangiectatic and sawdust livers by dietary means appears to be fully warranted. Since many of the hepatic lesions resulting from altered dietary cystine as noted by various authors (5,6,7) in the rat, are comparable to those observed in telangiectatic and sawdust bovine livers, a controlled dietary feeding experiment for the bovine species would seem indicated.

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THE SEPARATION OF THE RARE EARTHS ¹

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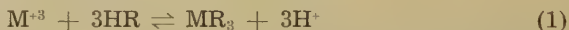
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Until the present time the separation of the rare earths from each other to produce pure salts has been a difficult task. Since the trivalent rare earths (yttrium included) exhibit the same chemical reactions, the separation techniques employed were those of fractionation. The synthetic organic resins having exchangeable cations have been employed recently in an ever-increasing number of processes, including the separation and preparation of pure rare earth salts.

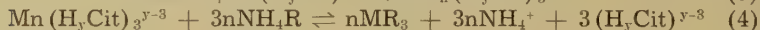
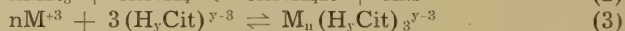
The process of separating the rare earths on an ion exchange resin column actually employs the principles of chromatography as well as ion exchange. With the resin in the hydrogen, or acid, cycle the rare earth ions will exchange with the hydrogen ions from the resin quantitatively. If the process is carried out in a medium at pH 1.8-2.0, with no more than small amounts of extraneous salts present, the full capacity of the resin at the top of the column is utilized. By passing a solution of citric acid at a carefully controlled pH through the column in a downward manner, the various rare earths are eluted at different rates, effecting a separation.

It can be shown that the efficiency of the separation is dependent upon a number of operating variables. The reactions which take place are quite reasonably assumed to be given in the following set of chemical equations.

The initial adsorption step is



where R is the anionic part of the resin and M is any trivalent rare earth ion. The elution step involves the continued repetition of the reactions



where M^{+3} is the uncomplexed rare earth ion, $(H_yCit)^{y-3}$ is some citrate ion whose exact composition is still undetermined, and n is restricted by the relation $n = 3 - y$. Since these processes occur a great number of times for each ion during the course of the elution, a separation of one rare earth species from another would depend upon the relative stabilities of the complex, $M_n(H_yCit)_3^{y-3}$, in solution.

The pH of the citrate solution controls the concentration of the

¹ Doctoral thesis number 937, submitted December 10, 1948.

(H_3Cit)³⁻ ion in equation (3), and the experimental evidence shows that equilibria in equations (3) and (4) are very important. Since the separation depends on the pH of the solution for any concentration of citric acid, the citric acid used as eluant was arbitrarily maintained at a concentration of 5 per cent (expressed as the monohydrate).

A systematic study was made of the factors which affect the separation. These include (1) the concentration of the citric acid, (2) the pH of the citric acid solution, (3) the length of the column, (4) the weight of the starting sample of rare earths, (5) the flow rate of the eluant solution, (6) the diameter of the column, (7) the composition of the starting sample, and (8) the mesh size of the resin. The particular resins used in the research were Amberlite IR-1 and Amberlite IR-100 which for all practical purposes have the same exchange groups and capacity, differing only in the physical stability of the polymeric networks.

The source of rare earths consisted of a synthetic starting material made up of cerium and yttrium. This pair of rare earths was tested both on tracer and macro amounts of material. The work with the neighboring pair of elements, neodymium and praseodymium, was done with only macro amounts of material. When it was demonstrated that a partial separation of neodymium from praseodymium could be effected, a large column (49 mm. diameter and 28 feet bed length) was erected to process, in a crude way, some commercial didymium to produce binary mixtures of the two species and enrich the praseodymium content by taking the appropriate cuts during the desorption process.

After investigating the above-mentioned operating variables systematically, the following results are presented:

1. *Citrate concentration.* Elutions were carried out at pH 2.75 using 2, 5, and 20 per cent citrate solutions. With the 2 per cent citrate, the material on the column was not desorbed. With the 20 per cent citrate, both rare earth components of the starting mixture were eluted together. The 5 per cent citrate solution at pH 2.75 did give partial separation with both tracer and macro amounts of cerium and yttrium. It is evident then that the choice of the citrate concentration is arbitrary and that there will exist a pH range over which a separation is effected for any particular concentration.

2. *pH.* The best pH values for separating a mixture of cerium and yttrium were in the region 2.60 to 2.65 pH for a 5 per cent citrate eluant. With the neighboring pair, neodymium and praseodymium, the best separation was obtained at a pH of 2.55. It was found that at pH values below 2.55 the time necessary for the elution increased rapidly.

3. *Length of column.* Increasing the length of column increases the separation, but also increases the length of time by a proportionate factor. In addition one must remember that the column is not operated at equilibrium conditions and that the leading and trailing edges of the individual elution bands become more sigmoidal in shape as the

band progresses down through the resin bed. An arbitrary value of 175 cm. for the resin bed lengths was chosen for the standard column.

4. *Weight of sample.* The maximum weight of material which can be used in any single elution process was found to be 0.5 g. of rare earth oxide per square centimeter of cross-sectional area of the column. A value between 0.25 and 0.50 g. per square centimeter is more advisable for columns having bed lengths of 175 cm.

5. *Flow rates.* The separation efficiency increased as the flow rate was reduced. At linear flow rates below 1 cm. per minute, the control of the flow rate becomes difficult for small columns. Change in flow rate has little effect on the volume of eluant necessary for breakthrough. The time factor for an elution varies inversely with the flow rate.

6. *Diameter of the column.* The columns employed in the work varied from 1 to 10 cm. in diameter. If the column is properly pre-conditioned, little change is noted in the separation from one column to the next.

7. *Composition of the starting sample.* The amount of pure material obtained during an elution depends to a large extent on the original composition of the mixture. This is not a variable, but nevertheless it must be considered as having an effect on the separation efficiency.

8. *Mesh size of the resin.* For the present work, a mesh size of 40-60 was used. Data from the literature indicate that the finer the particle size the more efficient the elution. However, operating conditions for the columns being used here require a steady flow rate and do not permit the fine resin size as it will pack together in the column and stop the flow of eluant.

The set of standard conditions so chosen was tested with a neodymium-praseodymium mixture containing 51.7 per cent Pr originally. More than 22.5 per cent of the neodymium was eluted with spectroscopic purity. With a neodymium-samarium mixture, more than 60 per cent of the samarium was eluted with spectroscopic purity. However, elutions of samarium-gadolinium and yttrium-dysprosium mixtures were not very efficient. Evidently considerably more work must be done with the heavier rare earths.

The order in which the rare earths are eluted progresses from lutecium to lanthanum. Yttrium presents itself between holmium and dysprosium. Due to the fact that the ionic radii of the hydrated rare earth ions decreases and the basicity increases in the same sequence, the elution order is explained.

NESTING AND PRODUCTION OF WATERFOWL
IN NORTHWEST IOWA: WITH SPECIAL REFERENCE TO
THE BLUE-WINGED TEAL (*ANAS DISCORS* LINNAEUS)¹

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With the inception of this project, four objectives were advanced: (1) to develop a method of determining yearly productivity prior to the hunting season, (2) to compare present productivity with past productivity on the same areas in an effort to determine waterfowl population trends, (3) to analyze some factors affecting waterfowl populations in Iowa, and (4) to recommend waterfowl management procedures for northwest Iowa.

Approximately 3,850 hours were spent in field observation in northwest Iowa during August and September in 1947, from March 14 to September 1 in 1948, and, except for an absence of 12 days during the middle of August, from March 18 to September 1 in 1949.

The majority of the spring migrants showed definite increases from 176,000 waterfowl (twenty-one species) recorded in 1948 to 220,000 waterfowl (twenty-three species) observed in 1949. A comparison of the migration data indicated the following spring arrival dates: blue-winged teal (*Anas discors*) and redhead (*Aythya americana*), last week of March; mallard (*Anas p. platyrhynchos*), middle of March; ruddy duck (*Erismatura jamiacensis rubida*), first week of April; and American coot (*Fulica a. americana*), third week of March. The sex ratio of 3,782 blue-winged teal sexed during spring migration was 1.5 males to 1 female. Blue-winged teal reached northwest Iowa 11 days later in the spring of 1949 than in 1948 with the flight peak for both years occurring about mid-April.

The 1948 estimated waterfowl breeding population was: blue-winged teal, 120 pairs; mallards, 20 pairs; redheads, 25 pairs; ruddy ducks, 40 pairs; and coots, 200 pairs. Except for the coots, there was an increase in 1949 in the breeding population of the above species. Approximately 140 pairs of blue-winged teal, 50 pairs of mallards, 25 pairs of redheads, 50 pairs of ruddy ducks, and 125 pairs of coots remained to nest in 1949.

Nesting and production data were obtained from observations on 278 waterfowl nests (blue-winged teal, 186; mallard, 26; redhead, 6; ruddy duck, 9; and coot, 51).

Blue-winged teal nested from the first week of May to the first

¹ Doctoral thesis number 1022, submitted December 14, 1949.

week of August, a period of about 100 days. Nest sites were selected by the female, and field observations on sixty-three indicated that nest building occurred from 7:00 to 10:00 A.M. More than 80 per cent of the teal nests were constructed and lined with old blue grass and down. Teal nests were found at distances varying from 20 to 210 yards from open water, with the mean distance for 186 nests being 79.35 yards. Measurements taken on 186 nests indicated the following means: outside diameter, 7.7 inches \pm .56; inside diameter, 5.3 inches \pm .78; bowl depth, 2.2 inches \pm .58; and thickness under bowl, 0.8 inches \pm .38. Measurements of 142 blue-winged teal eggs gave a mean width of 33.9 ml. \pm .37 with a range from 30 to 37 ml., and a length of 47.1 ml. \pm .75 with a range of 38 to 51 ml. Egg laying took place between 7:00 and 10:00 A.M. The mean number of eggs per active nest was 7.9 and per destroyed nest, 5.4. Incubation apparently began within 24 hours after the last egg was deposited and continued for 21 to 23 days.

In 1948, 23.0 per cent of the teal nests hatched successfully and in 1949, 21.4 per cent. The mean number of eggs hatched from normal nests of the following species for 1948 and 1949 was: blue-winged teal, 9.32 (40 nests); mallard, 10.6 (5 nests); redhead, 9.66 (3 nests); ruddy duck, 6.75 (8 nests); and coot, 6.6 (30 nests). Fifty-two of 186 teal nests were judged to be renesting attempts with fourteen (26 per cent) hatching successfully. The teal nesting density for 1,611 acres of blue grass was one nest per 12.2 acres and for 473 acres of sedge-meadow, one nest per 11.5 acres. The heaviest concentration, one teal nest per 1.3 acres, occurred on the 30-acre ungrazed island in Barringer's Slough, 1949.

Three cover types (blue grass, sedge-meadow, and hay fields) were examined for nesting teal, but nests were found in only two: blue grass, 145 and sedge-meadow, 41. Application of analysis of co-variance indicated no significant statistical difference ($F = 3.21$, .05 per cent = 9.28) between extent of cover types and number of nests, or that blue grass cover was utilized to a greater extent than sedge-meadow cover ($F = 0.024$, .05 per cent = 200). Within a utilized cover, nesting density appeared to be determined by availability rather than cover preference ($X^2 = 0.09$, .05 per cent = 3.841).

Ecological measurements made to determine cover effects on nest success included: cover extent, cover type, cover height, light intensity, crown-area extent, cover density, stem density, topographic location, and height above water level.

Examination of the data by analysis of variance indicated that neither extent of nesting cover ($F = 4.35$, .05 per cent = 19.16) nor cover type ($F = 0.01$, .05 per cent = 200) were significant factors in determining the fate of teal nests. There was a significant statistical difference ($F = 13.74$, .05 per cent = 10.13) between successful and unsuccessful nests and the stem density. Seventy per cent of the successful nests occurred in cover which was classified as either sparse (0-299 stems/sq. ft.) or light (300-499 stems/sq. ft.). Further analysis

indicated a significant difference ($F = 5.52$, .05 per cent = 5.05) between successful and unsuccessful nests with respect to light intensity at the nest in the two cover types. No significant statistical differences were found between successful and unsuccessful nests with respect to the other ecological measurements (cover height, $F = 4.65$, .05 per cent = 18.51; crown-area, $F = 1.541$, .05 per cent = 10.13; cover density, $F = 5.329$, .05 per cent = 18.51; topographic location, $F = 8.80$, .05 per cent = 10.13; and height above water level, $F = 4.35$, .05 per cent = 7.71). The biological implication of these data indicated that the major portion of the teal nests tended to be located in cover of low light intensity (0-10 per cent) and that more of these nests terminated successfully than those located in open cover. The hypothesis that adequate nesting cover, possibly that of low light intensity with a light to sparse stem density (0-499 stems/sq. ft.) tended to form good concealment for nesting teal and increased the number and success of nests, may be applicable in this situation.

The detrimental effects of burning were reflected in teal nesting populations as much as 1 year after the fire. Predation was responsible for the loss of 100 of 146 destroyed teal nests. Parasitism and human interference were minor factors affecting nest success.

More waterfowl nested with greater success in lightly or ungrazed cover than in medium or heavily grazed cover.

The mean brood size at the end of the rearing season was: blue-winged teal, 5.16 (42 broods); mallard, 7.16 (6 broods); redhead, 7.42 (14 broods); ruddy duck, 3.62 (47 broods); and coot, 3.64 (48 broods).

The 1948 calculated number of juveniles produced per breeding pair was: blue-winged teal, 1.33; mallard, 2.8; and coot, 2.2. The 1949 calculated number of juveniles produced per breeding pair was: blue-winged teal, 1.35; mallard, 1.2; redhead, 4.4; ruddy duck, 1.9; and coot, 1.7.

Recommended management practices to increase waterfowl nesting include: (1) protecting a 150-yard margin of vegetation around water areas from overgrazing and fire, (2) spraying noxious weeds and patches of forbs with herbicides, (3) removing plants where herbaceous vegetation dominates and then reseeding with blue grass, (4) maintaining present nesting areas, (5) educating youth in trapping techniques and fur utilization, and (6) acquiring and restoring as much of the north-western Iowa pothole country as is economically and financially possible by agencies interested in waterfowl conservation.

REACTIONS OF BIOLOGICAL ORGANIC PHOSPHORUS COMPOUNDS WITH CLAYS¹

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An investigation was conducted to obtain information on the reactions of clays with a variety of biological organic phosphorus compounds.

Adsorption of mononucleotides, nucleic acids and nucleoprotein by bentonite, illite and kaolinite depended on the type and concentration of clay and nucleic acid material, the pH, the inorganic cations present, the time of reaction and the temperature of the system. Increasing the concentration of nucleic acid material and decreasing the pH or the concentration of clay increased adsorption per gram of clay. The sequence of decreasing adsorptive capacity was bentonite > illite > kaolinite and of decreasing reactive capacity was nucleic acids and nucleoprotein > mononucleotides. Adsorption was greater with calcium and magnesium than with sodium and potassium as exchangeable inorganic cations. The adsorption reaction for ribonucleic acid could be fairly

well defined by the equilibrium equation
$$K = \frac{(\text{Clay-H})(\text{Nucleate})^-}{(\text{Complex})^-}$$

The rate of reaction of ribonucleic acid with clays was initially rapid but became much slower as equilibrium was approached (48 to 96 hours). Increasing amounts of exchangeable bases decreased the rate of reaction and increasing concentrations of ribonucleic acid increased the rate of reaction. An increase of 30 to 40°C. increased the rate of reaction but did not change the position of equilibrium. The reaction was found to be slowly but completely reversible at low, intermediate, and high pH values. Repeated extraction of the bentonite-ribonucleic acid complex with water or 1N sodium chloride removed small portions of nucleic acid. One extraction with 1N hydrochloric acid removed a large amount but not all the nucleic acid. Nearly all the nucleic acid was rendered soluble by single extractions with buffer solutions above pH 8.

Increasing concentrations of sodium chloride increased the rate of reaction of ribonucleic acid with clays, decreased the pH values of the suspensions, and increased equilibrium adsorption. The finer fractions of illite and kaolinite adsorbed more ribonucleic acid than did the coarser fractions in the absence or presence of sodium chloride. No

¹ Doctoral thesis number 1071, submitted June 5, 1950.

differences in adsorption by coarse, medium, and fine fractions of bentonite could be detected.

Relatively high concentrations of amino acids decreased adsorption of ribonucleic acid by bentonite. Increasing concentrations of gelatin decreased adsorption to a much greater extent than did amino acids. Adsorption of ribonucleic acid by zein depended on the concentrations of nucleic acid and zein and on the pH values of the systems in a similar manner to clays.

X-ray studies of bentonite-nucleic acid complexes indicated that the organic materials were arranged within the expansible portion of the clay lattice in such a manner as to allow minimum possible distances between the silicate layers when the samples were dried.

Adsorption of carbohydrate phosphates by bentonite appeared to be similar in principle to adsorption of inorganic phosphorus but varied greatly in degree. The sequence of decreasing adsorption was sodium phytate > sodium phytate derivatives > varium fructose 1-6 diphosphate > varium fructose-6-phosphate > potassium dihydrogen phosphate > potassium glucose-1-phosphate > sodium glycerophosphate > barium phosphoglycerate. Adsorption was small or negligible above pH 7 and usually at a maximum between pH 5 and 6, except for sodium phytate and its derivatives for which maximum adsorption took place between pH 3 and 4. Very little barium phosphoglycerate was adsorbed and sodium glycerophosphate was adsorbed to a lesser extent than was calcium glycerophosphate.

Adsorption of carbohydrate phosphates by fine and coarse fractions of bentonite was similar. Adsorption by fine and coarse fractions of illite was small and of kaolinite negligible. A Shelby subsoil with free iron oxides removed fixed about half as much fructose 1-6 diphosphate as did the same material without free iron oxides removed. The presence of citric acid reduced the fixation of phytin by bentonite to about one-tenth the amount that took place in an acetate buffer. Adsorption from low concentrations of carbohydrate phosphates by kaolinite and bentonite did not generally take the form of a Freundlich adsorption isotherm and if so only fortuitously.

Adsorption of lecithin and cephalin from water solution was small or negligible but considerable in the presence of organic solvents. In water solution adsorption per gram of clay appeared to be uninfluenced by clay concentration but decreased with increasing pH. In ethanol, ethanol-ethyl ether and water-ethanol-ethyl ether solutions, adsorption increased with decreasing concentration of clay and/or increasing concentration of phospholipid, and decreased with increasing pH. Furthermore, in water-ether-alcohol solution adsorption of phospholipids by bentonite was considerably greater than by kaolinite.

The separation of the phosphorus fractions of fresh microbial tissue, autoclaved tissue and tissue in the presence of increasing concentrations of kaolinite and bentonite was investigated. Autoclaving or autolysis for several days resulted in degradation of the material which made it

impossible to obtain clearcut separations. The presence of clays markedly influenced the distribution of the various phosphorus fractions and bentonite was considerably more effective than was kaolinite. The nucleic acid fractions seemed to be increased at the expense of the water soluble, acid soluble, and phospholipid fractions. On degradation of fresh tissue the acid soluble organic phosphorus fraction decreased at the most rapid rate, but in the presence of clays most of the losses appeared to take place from the apparent nucleic acid fraction.

The distribution of the phosphorus fractions of degraded microbial tissue accumulated in sand-bentonite mixtures containing different amounts of bentonite was essentially the same as fresh microbial tissue in the presence of bentonite, except that all the inorganic phosphorus was not extracted by the water and acid treatments used, and the relative amounts of phospholipids appeared to increase with increasing quantities of clay.

Dephosphorylation of microbial phosphorus decreased with decreasing pH and increasing clay concentration, and bentonite decreased mineralization more than did kaolinite. At low values pH was the dominant factor influencing mineralization, but between pH 6 and 7 clay concentration appeared to exert a relatively greater effect than hydrogen ion concentration on the mineralization of microbial organic phosphorus.

Extraction of soil organic phosphorus showed that it was least soluble between pH 1 and 4. Its solubility increased slowly from pH 4 to 7, more rapidly from pH 7 to 8 and very rapidly above pH 8. Maximum insolubility of soil inorganic phosphorus was between pH 6 and 7 for the samples studied.

CENTER OF FLEXURE OF BEAMS OF TRIANGULAR CROSS-SECTION¹

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The resolution of Saint-Venant's general problem of a loaded cantilever beam into the simpler problems of torsion and pure flexure involves the determination of a load point P in the end section such that the local twist vanishes at a point Q in the section. The position of P in general depends upon the position of Q , the choice of which in turn is made in any convenient manner. For most problems the preferred position of Q is at the centroid, in which case P is called the "center of flexure."

The center of flexure coincides with the centroid in biaxially symmetric sections. It has previously been determined for a few sections of uniaxial symmetry and for one asymmetric section. Among triangular cross-sections it is known only for the isosceles right triangle and for the equilateral triangle when Poisson's ratio is equal to one-half, the result in the latter case being obvious from symmetry. In addition the center of flexure has been determined experimentally for a beam whose cross-section is a narrow isosceles triangle (1).

In this thesis the center of flexure is found for any isosceles triangle and for the general triangle with Poisson's ratio equal to one-half, the direction of the load being arbitrary. A line of flexure is found for any right triangle when the load acts parallel to the hypotenuse. In these cases the results are such that, if none of the angles is small, the position of the center of flexure for the completely general triangular section may be inferred. All of the previously known solutions, including the one obtained by experiment, appear as special cases of the result for the isosceles-triangular section.

The origin of coordinates is taken to be in the fixed end of the beam, with the z -axis parallel to the longitudinal edges. The loaded end is in the plane $z = h$. The coordinates (x_o, y_o) of the center of flexure are determined from the equation

$$\iint_S (\chi \tau_{yz} - y \tau_{zx}) dx dy = \chi_o (W_y + \frac{W_x P_{xy}}{I_y}) - y_o (W_x + \frac{W_y P_{xy}}{I_x}),$$

which expresses the fact that the resultant torsional couple due to the stress components τ_{yz} and τ_{zx} is equal to the moment about the z -axis of the load applied at (x_o, y_o, h) . In this equation, which is an identity in

¹ Doctoral thesis number 1032, submitted March 9, 1950.

W_x and W_y , the coefficients of x_0 and y_0 are the components of the load parallel to the y - and x -axes respectively. The moments and product of inertia are referred to axes through the centroid of the cross-section parallel to the coordinate axes. The stress components τ_{yz} and τ_{zx} are constructed as usual in Saint-Venant's flexure problem to satisfy the stress-equilibrium equations, the equations of compatibility, and a boundary condition which insures that the lateral surface of the beam is not acted upon by external forces. The satisfaction of the last condition involves the determination of a flexure function unique for the particular cross-section. Flexure functions for the three cases under consideration herein have been found by Seth (2).

For the cross-section in the form of an isosceles triangle, the center of flexure lies on the axis of symmetry at a distance y_0 from the vertex on this axis given by the formula

$$(a) \quad y_0 = \frac{2d\{5[(1+\sigma)\tan^2\beta - \sigma] - 2(1-2\sigma)\}}{5(1+\sigma)(3\tan^2\beta - 1)} - \frac{3(1+\sigma)\tan^4\beta - 3\tan^2\beta + \sigma}{(1+\sigma)\tan^3\beta(3\tan^2\beta - 1)} \frac{D}{\mu d^3},$$

in which d is the altitude from the vertex, 2β is the angle at the vertex, σ is Poisson's ratio, μ is the rigidity modulus, and D is the torsional rigidity. For the general triangle, the equations of whose sides are $y = d$, $y = m_1x$, $y = m_2x$, the coordinates of the center of flexure are found to be, when $\sigma = 1/2$,

$$(b) \quad x_0 = \frac{d(m_1 + m_2)}{3m_1m_2} - \frac{4(m_1 + m_2)(2m_1^2 - 5m_1m_2 + 2m_2^2 - m_1^2m_2^2)}{3(m_2 - m_1)^3} \frac{D}{\mu d^3},$$

$$y_0 = \frac{2d}{3} - \frac{2m_1m_2(m_1^2 - 4m_1m_2 + m_2^2 - 2m_1^2m_2^2)}{3(m_2 - m_1)^3} \frac{D}{\mu d^3}.$$

For the right triangle with vertices at $(0, 0)$, $(0, c)$, $(-c/m, 0)$, when the load acts parallel to the hypotenuse, the coordinates of the center of flexure are connected by the equation

$$mx_0 - y_0 = \frac{4\sigma Dm(m^2 + 1)}{\mu c^3(1 + \sigma)} - \frac{c(3 + 4\sigma)}{5(1 + \sigma)}.$$

Each of the above formulas contains as an unknown factor the torsional rigidity D , which must be evaluated before the solution can be considered complete. This quantity is known exactly for the equilateral and isosceles right triangles and can be approximated for other cross-sections by any one of several well-known methods. For the isosceles triangle an approximation deduced by the Rayleigh-Ritz method by Duncan, Ellis, and Scruton (1) is already known. The same method is used in this thesis to obtain an approximation for the right-triangular

section, and for the general triangle the approximation of Saint-Venant is employed.

When the values of D and β for the equilateral triangle are used in (a), it is found that for σ different from one-half, y_o has the limiting value

$$y_o = \frac{11 + 8\sigma}{15(1 + \sigma)} d.$$

This result has significance only when the boundary condition on two of the sides is relaxed to the extent of allowing the resultant of the external forces to vanish.

With the appropriate values of D , it is found that in the case of the isosceles triangle the influence of σ upon the position of the center of flexure is negligible except when the angle β is small, and that the line of flexure in the right triangle is completely independent of σ . It is concluded that the coordinates of the center of flexure for the general triangle and arbitrary σ are therefore reasonably well approximated by the formula (b).¹

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SOME FACTORS INFLUENCING THE SLICING QUALITY AND THE PALATABILITY OF CANNED BEEF¹

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The long processing period at high temperature to which beef is subjected during canning tends to adversely affect the tenderness, texture, and juiciness of the meat. The fibers are often rather tough, stringy, and dry, and the meat does not slice well.

This study was made to determine the effect on the slicing quality, the palatability, and the microscopic appearance of canned beef of injecting the raw meat with a solution of one of the following: sodium chloride, sodium chloride and lactic acid, or lactic acid. Each of the solutions containing sodium chloride was made to 15 per cent strength, which gave the proportion of approximately 1.5 grams of salt to 100 grams of beef after injection. The solutions containing lactic acid or the mixture of sodium chloride and lactic acid contained sufficient acid to give a pH of 3.4 in the solution.

Paired muscles were separated from the carcasses of four beef type steers (carcass grade Commercial) and from one aged dairy cow (carcass grade Cutter). The rib and loin portions of longissimus dorsi, psoas major and psoas minor, semitendinosus, semimembranosus, and biceps femoris muscles were each divided into three or six cuts, depending on the size of the muscle. Cuts from the right side were injected 1 day after slaughter of the animal and those from the left side were used as controls. After aging 8 days at 34° to 36°F., the cuts were canned. Processing was at 240°F. (10 pounds) for 65 minutes for the meat from three of the steers; for 90 minutes for the meat from the fourth steer and the cow.

The canned meat was sliced on a mechanical slicer and representative slices from each can were scored by three judges for aroma, flavor, tenderness, juiciness, and texture. The liquid from the can was scored for flavor. Slicing quality of the canned meat was evaluated by: (1) count of number of slices obtained, (2) weight of unsliceable meat, and (3) judges' scores for sliceability. Judges' scores for either palatability or slicing quality were on the basis of a 10-point scale (10 high, 1 low). The scores for flavor of meat, tenderness, juiciness, texture, and sliceability were analyzed statistically. Histological sections were made of the rib portion of the longissimus dorsi muscle. Other data included pH values and weight changes.

The results indicated little or no difference in the average aroma scores between injected and uninjected samples of canned beef for any

¹ Doctoral thesis number 990 submitted August 18, 1949.

of the three injection treatments. Muscle differences were negligible, but aroma scores were lower for the meat of the aged dairy cow than for the meat of the steers.

The flavor, tenderness, and texture of the beef injected with either sodium chloride solution or the mixture of sodium chloride and lactic acid were judged to be significantly higher (.01 level) for the injected than for the control samples. Lactic acid injection had little effect on these three palatability factors. No significant differences in flavor among the muscles were shown by judges' scores, but the flavor of the meat from the dairy cow was undesirably strong. Tenderness was influenced by the injecting treatments and variations were found among muscles and among animals. Texture differed in muscles and in animals. The psoas muscles had the highest texture rating; the loin portion of the longissimus dorsi and the semimembranosus, the lowest. Beef from the dairy cow had a low texture rating.

The average juiciness scores for the sodium chloride-injected samples of canned beef processed 65 minutes were higher than for the controls, but the differences between the juiciness scores for the injected samples processed 90 minutes and the control samples were not significant. Combined salt and lactic acid solution improved the juiciness of injected samples compared to uninjected samples (significant at .05 level). Lactic acid injection had little effect on the juiciness of the meat.

The flavor of the liquid from the can was improved by treatment of beef with either of the salt-containing solutions, but only slight differences in injected versus control samples resulted from lactic acid treatment.

The slicing quality of the canned beef was little affected by injection of the raw meat with any of the three solutions tested. Little variation in sliceability occurred among the cuts processed 65 minutes; among those processed 90 minutes, the beef from the cow sliced better than that from the steer. Muscles differed significantly in slicing quality. The semitendinosus sliced better than any of the other muscles.

The histological characteristics observed in the longitudinal sections of the rib portion of the longissimus dorsi were related to the palatability findings. Disintegration of fiber striations had occurred after 8 days of aging, but the extent of disintegration varied with the muscle and the animal. Injection of the beef with either sodium chloride solution or a mixture of sodium chloride and lactic acid resulted in an increase in the number and extent of disintegration fissures in the muscle fibers. This disintegrated appearance was associated with high tenderness rating by the judges of injected versus control samples. Samples injected with only lactic acid were similar to the control samples in histological characteristics.

The pH values varied little for the fresh muscles and for the cuts of beef aged 8 days regardless of treatment by injection. These average values for the five animals were close to pH 5.45. The meat of the aged dairy cow was higher in pH than the meat of the steers; canned meat

had higher values than the raw beef. Injection with any of the three solutions had little effect on pH values of the canned meat.

Small weight losses were found in the control cuts during aging but the average weight of injected samples was higher after 8 days of aging than the initial weight. The canned beef was approximately two-thirds meat and one-third liquid. Injection of the raw beef made little difference in the proportion of liquid to meat in the canned product.

It was concluded that under the conditions of this study:

1. As indicated by palatability scores, injection of beef cuts with either sodium chloride solution or a combination of sodium chloride and lactic acid markedly improved the flavor, tenderness, and texture of the canned beef compared to uninjected cuts. Juiciness was improved for some cuts but not for all. Aroma scores were little affected by the injection treatment of the meat; flavor of the liquid from the can was improved by injection of the beef with the salt-containing solutions but not with the lactic acid solution.

2. The slicing quality of the canned meat was fairly low and no improvement was obtained by injection of the raw meat with any of the three solutions tested. This quality varied, however, among the muscles and the animals.

3. The tenderness of the canned beef was related to the microscopic changes which occurred in the beef.

THE INFLUENCE OF OXYGEN AND CARBON DIOXIDE LEVELS IN THE SUBSTRATE UPON POTASSIUM ABSORPTION BY PLANTS¹

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The physiology of plants is known to be influenced by the state of aeration (oxygen and carbon dioxide levels) of the substrate. More specifically the aeration status of certain soils in Iowa has been found to influence to a marked degree the growth and potassium nutrition of corn. Little is known however concerning the interrelationship of oxygen, carbon dioxide, and nitrogen percentages and for their controlled in the field.

This investigation was made to determine the influence of various oxygen and carbon dioxide levels in soils and nutrient cultures upon the growth, water absorption, and potassium nutrition of plants. A special apparatus was designed for the preparation of gases of varying oxygen, carbon dioxide and nitrogen percentages and for their controlled flow through soils and nutrient cultures.

In one soil aeration experiment where pure carbon dioxide at a rate of 10 liters per 24 hours was passed through the soil in 1-gallon pots, the growth of corn roots was largely limited to the upper half of the pot. The soil atmosphere at approximately the depth of root penetration was 30 to 40 per cent carbon dioxide and about 10 to 12 per cent oxygen. Corn roots penetrated throughout the soil in pots aerated with 100 per cent nitrogen. The soil atmosphere in this case contained 15 to 16 per cent oxygen and less than 1 per cent carbon dioxide.

Corn and soybean plants 6 weeks of age were used in a potassium and water absorption study in which the aeration period was 72 hours. The tops of the pots were closed with a paraffin-vaseline mixture and different soil air compositions were obtained by aerating with various gas mixtures at a rate of 10 liters per pot per 24 hours. With aerating gases containing 20 per cent oxygen and variable amounts of carbon dioxide, the potassium uptake of corn and soybeans was not reduced until the carbon dioxide was increased to 20 and 50 per cent. Water absorption was not reduced until the carbon dioxide was increased to 50 per cent. Aeration with pure nitrogen gas, which reduced the oxygen in the soil atmosphere to about 1 to 6 per cent, had no effect on potassium and water absorption. This indicates that in a constantly streaming soil atmosphere the oxygen needs of the plant may be supplied when the oxygen percentage is very low, but that at a carbon

¹ Doctoral thesis number 1019, submitted December 13, 1949.

dioxide concentration of around 20 per cent a reduction in potassium uptake may occur even when adequate oxygen is present.

The growth of corn in 1-gallon pots of soil, in which the aeration was restricted by closing the pots with a sheet of parafilm, was greatly reduced even though the soil atmosphere was found to contain around 17 to 18 per cent oxygen and 2 to 3 per cent carbon dioxide. The composition of the soil atmosphere apparently does not give a true picture of the aeration status of the soil, especially when a comparison is made between static and streaming soil atmospheres. The immediate gaseous environment of the roots cannot be known accurately in a static atmosphere when the sample of air is as large as 10 milliliters.

In order to control the immediate environment of the root and to obtain a better measure of the potassium absorption by the plants, culture solutions were used in further aeration studies. Corn plants were grown in quart Mason jars of Hoagland's nutrient solution for a period of from 3 to 6 weeks, after which uniform plants were selected for a 5-day aeration treatment. Various gas mixtures were passed through the nutrient solution at the rate of 20 liters of gas per jar per 24 hours. During the period of aeration samples of the nutrient solution were obtained for potassium analysis. The loss in potassium from the solution indicated the amount of potassium absorbed by the plants.

These culture solution studies gave results in which the effects of the different aerating gases on potassium and water absorption were much more pronounced than when soils were used. There was a reduction in both potassium and water absorption when the aerating gases were 100 per cent nitrogen, 20 per cent oxygen plus 20 per cent carbon dioxide, and 20 per cent oxygen plus 50 per cent carbon dioxide. Ten per cent carbon dioxide in the aerating gas was without effect on these processes when 20 per cent oxygen was also present.

When the oxygen percentage in the aerating gas was very low (0.4 per cent) the addition of 5 per cent carbon dioxide reduced the potassium and water absorption of corn still further. This seemed to indicate that the detrimental level of carbon dioxide in the substrate depends upon the amount of oxygen present. There was also evidence of an additive effect of carbon dioxide over an oxygen deficiency when corn plants were aerated for a period of 3 weeks and harvested for dry weight yields. These yields were lower where 20 per cent carbon dioxide was added to purified nitrogen in the aerating gas than where purified nitrogen alone was used. The additive effect of the carbon dioxide was also evident in a 5-day aeration period on the water absorption by corn, but was not so marked on the uptake of potassium.

Reduction of the oxygen in the aerating gas with an equal increase in carbon dioxide did not markedly reduce growth and potassium absorption of corn until the oxygen was reduced to 5 per cent and the carbon dioxide increased to 15 per cent. However, the growth and potassium absorption was somewhat reduced below the value for 20

per cent oxygen when the aerating gases contained 15 per cent oxygen plus 5 per cent carbon dioxide, and 10 per cent oxygen plus 10 per cent carbon dioxide.

In general these results indicate that carbon dioxide has a specific detrimental effect on growth and potassium and water absorption of plants, and that the level of carbon dioxide necessary for these effects depends upon the amount of oxygen present near the root surface.

The soil air composition values of field plots on which different tillage practices had been used were not correlated with the tillage practice or with the growth response of corn, and did not deviate greatly from the composition of the atmosphere. It was concluded that the composition of the soil atmosphere may not indicate the true aeration status of the soil since low concentrations of oxygen and high concentrations of carbon dioxide could probably exist in the smaller soil pores and next to the root surface without being detected in a 10-milliliter sample of soil air.

One greenhouse experiment was devoted to a study of two oxygen-carrying chemicals, barium peroxide and ammonium persulphate, as possible sources of oxygen for plants growing under water-logged conditions. Significant increases in the yield of corn were obtained with ammonium persulphate, but the yields were still far from normal for a well aerated soil. The results indicate that possibilities for successful oxygen fertilization do exist.

A complete description of the aeration apparatus, techniques used for soil air analyses, photographs, and tables of data are given in the thesis.

INTERACTIONS IN GRASS-LEGUME PLANTINGS¹

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Data were taken from experimental plots at the Agronomy Farm, Ames, Iowa, and the College Pasture Improvement Farm, Albia, Iowa, designed to test the interactions of grass-legume plantings as measured by yield, protein percentage, and carotene content. Four grasses, timothy, bromegrass, orchardgrass, and Kentucky bluegrass, were seeded alone and in mixtures with each of three legumes, Ladino clover, alfalfa, and birdsfoot trefoil, at the Albia station in April of 1945. Ladino clover at Albia was completely winter-killed in the winter of 1947-48. The four grasses, and in addition reed canarygrass and alta fescue, and the three legumes were seeded alone and in all combinations of a grass and a legume at the Agronomy Farm in the spring of 1947. The data were obtained during the growing season of 1948.

Grasses and legumes grown together may be superior to pure stands of either component for pasture or hay. The legume component adds yield and protein to the mixture. The grass adds bulk and carbohydrate, and increases soil binding.

In the experiment at Ames, the average yield of grass-legume mixtures was 90 per cent as much as legumes alone, and grasses alone 65 per cent as much as legumes alone. Alfalfa alone was the highest yielding crop with 3.02 tons of forage, followed by timothy-alfalfa, orchardgrass-alfalfa and bromegrass-alfalfa with yields of 2.68 to 2.55 tons.

Alfalfa was decidedly superior to trefoil and still more superior to Ladino in yield, although the latter legumes improved toward the end of the season. Bromegrass and timothy were the better grasses and bluegrass the poorest in yield. Reed canarygrass started slowly, but was one of the high ranking grasses at the second harvest.

The stands at Albia were seeded in 1945 and were in their fourth season in 1948. On this heavy, low-nitrogen soil, grasses alone were poor, and grass-legume mixtures outyielded grasses by nearly four times. Legumes planted alone were not available for comparison. The bromegrass-trefoil mixture was high yielding with 3.12 tons of forage compared with 0.62 tons for bromegrass alone. Alfalfa was the second best legume on this soil and timothy the second best grass.

Continued association with a legume may increase the per-acre yield of grass by 100 per cent, in addition to the legume growth. This

¹Doctoral thesis number 1050, submitted May 24, 1950.

gain appears to be due to a build-up of nitrogen from sloughed legume tissues or residue.

Increased protein percentages in grasses grown with legumes might be due to: (a) shading and reduced nonprotein material in the grasses; (b) gradual accumulation of nitrogen from legume residues; or (c) direct transfer of soluble nitrogen from functioning legume nodules to associated grass roots. Results, both at Ames and at Albia, agree with those of many previous workers in suggesting higher protein in grasses grown in association with a legume when total nitrogen on a dry weight basis is used as a measure of protein percentages. On a green weight basis, however, differences tended to disappear. Such a shift indicates that the high protein percentages in grasses grown with legumes were in part only low dry matter percentages, so that a pound of dry forage represented more plant material and more protein. Later in the season at Ames, however, there were evidences of real gains in nitrogen, particularly in grasses grown with alfalfa, which could be attributed either to nitrogen transfer or to sloughing and decomposition of legume nodules. Transfer by sloughing seems the more probable reaction.

In the one Albia harvest in which green weights were available, all of the protein gains were assignable to differences in dry matter. In some harvests at this station, added available nitrogen was utilized in greater growth until no gain in protein percentage was observable on any basis, although total protein per acre in the grass was doubled by association with a legume.

Carotene percentages were measured throughout the season on alfalfa and brome-grass at Ames only. Carotene contents of the two plants were nearly equal. Carotene decreased with maturity of brome-grass, but increased with the season in aftermath of comparable maturity. Brome-grass grown with alfalfa was significantly higher in carotene than brome grown alone.

Greenhouse experiments were set up with barley alone or with peas, and brome-grass alone or with alfalfa in glazed 1-gallon pots. A temperature of 45° to 60°F. was maintained and normal photoperiods of about 10 hours or photoperiods of 20- to 24-hours were used in an attempt to obtain conditions favorable for direct transfer of nitrogen from legume to grass. Both the grasses and legumes made a much greater total growth on the long photoperiods. The greenhouse experiments showed highly significant increases in protein of barley grown with peas when compared on a dry weight basis, but no difference at all on a green weight basis. This experiment was designed to give optimum conditions for direct transfer of nitrogen from legume to grass. No satisfactory evidence of a direct transfer of nitrogen was obtained.

GENETIC VARIATION AND COVARIATION IN TYPE AND BUTTERFAT PRODUCTION AMONG JERSEY COWS ¹

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The genetic and environmental relations underlying a phenotypic correlation between type and production must be known in order to construct an index that will maximize the amount of genetic progress when selecting for both traits. Estimates of the necessary phenotypic and genetic parameters were obtained by analyzing the official type ratings and fat production records of 8,464 Jersey cows who were from 245 herds. These herds were on Herd Improvement Registry test for at least 4 of the 5 years, 1943 to 1947 inclusive.

Genetic variation constituted 18 per cent of the intraherd and intra-year variance of single records of fat production and 14 per cent of the intraherd variance of type ratings. The genetic correlation between these two characters was estimated as .18 from 2,786 daughter-dam pairs, .12 from the paternal sister analysis, .21 from the maternal sister analysis, and —.26 from the 519 sets of full sisters. The paternal and maternal sister analyses contained the same 8,464 cows. The estimate of the genetic correlation from the daughter-dam pairs was considered to be the most reliable.

The intraherd phenotypic correlation between type and a single production record of the same cow was .12. Only about 15 to 30 per cent of this correlation was caused by genes which affected both type and production alike; the remainder being caused by environmental variations which affected both in the same direction.

On the basis of single records, the correlations between the phenotypes of half and full sisters were as follows:

Correlation	Half Sisters		Full Sisters
	Paternal	Maternal	
$r_{TT'}$.14	.12	.16
$r_{PV'}$.12	.08	.09
$r_{TP'} = r_{PT'}$.02	.03	— .04

In view of the estimates of heritability for type and production from the daughter-dam analysis, the environmental contribution to $r_{TT'}$ and $r_{PV'}$ for paternal half sisters was more important than the genetic. The same was true for the likeness between the type ratings of maternal

¹ Doctoral thesis number 1014, submitted December 12, 1949.

sisters. $r_{TT'}$ and $r_{TP'}$ for full sisters were about as expected considering the heritability estimates from the daughter-dam analysis and the expected environmental contributions. $r_{TP'}$ was less than expected for full sisters but the degrees of freedom are few enough that sampling errors could account for this discrepancy.

Differences in average type from one herd to another accounted for 16 per cent of the total variance in type ratings. Differences between herds were considerably more important in the total variance of average production, accounting for 40 to 45 per cent of that. No effort was made to ascertain the importance of heredity and environment in causing the differences between herds.

The fraction of the intraherd variance in average production (average of two records per cow) due to yearly changes in the general environment was only 4.1 per cent. The yearly effects on production are largely eliminated when the average of all records on the cow is studied. The amount remaining in the average of n records will be about $1/n$ of what it was for single records. The distribution of these yearly effects with respect to the mean squares used to determine correlations between the average production of paternal and maternal sisters or full sisters in dairy data is such that they can usually be ignored; unless one is interested in the magnitude of the various components rather than the ratio which is necessary to calculate the correlations.

A comparison of the average type rating of 2,044 dams, which had a daughter with one or more records of production, with the average type of all 8,464 cows indicated that, in general, breeders were giving some attention to type in their selection program. Proportionately, the difference between the averages of dams and daughters was larger for type than production. Two possible explanations were given for this difference: (1) the dams were older than the daughters and age differences were not removed from the type ratings, and (2) breeders actually may have been giving more attention to type than production in their selection of the dams.

Using the heritability estimates, genetic correlation, and phenotypic correlations obtained in this study, two selection indexes were developed by Hazel's multiple regression technique; first, by giving type one-third as much attention as fat production and, second, by giving both characters equal attention. Only information about the phenotypes of the dam and her daughter were considered in constructing these indexes. Although selection on the basis of type alone should automatically bring about some genetic improvement in production, it would require about six generations to obtain the improvement that selection on the basis of production would obtain in one generation. The most efficient gain is obtained by using a selection index that has been constructed properly and utilizes all information that might be available on both type and production.

RADIOACTIVITIES PRODUCED IN EUROPIUM AND GADOLINIUM ¹

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The studies of radioactivities produced in gadolinium and europium were undertaken with the following objectives in mind. The determination of half-lives, mode of decay, energy of emitted radiations, and decay schemes of gadolinium activities, and the assignment where possible to the proper isotope were the primary objectives. The accomplishment of these aims increases the knowledge concerning new nuclides and provides the necessary information so that these tracers may be used in rare earth separations to identify the gadolinium fraction. Also the studies were designed to clear up debated points in regard to europium activities. It was hoped that some assignment of radiations and half-lives could be made by the comparison of deuteron- and neutron-produced europium activities. Primarily, this investigation deals with long-lived activities although two short-lived activities were studied.

Gadolinium oxide purified by cation exchange methods was loaned for this investigation by the rare earth group of this laboratory. Spectrographic analysis indicated the presence of samarium (0.28 per cent) and possibly terbium, yttrium, dysprosium, and holmium, but the amounts of these contaminants could not be estimated.

The gadolinium activities were purified after bombardment by sodium amalgam extractions to eliminate any possible europium or samarium activities. Cation exchange experiments with Nalcite high-capacity resin separated the rare earth contaminants that could not be extracted in the sodium amalgam. The eluting agent, resin preparation, identification, and amount of rare earth contaminants in the Gd_2O_3 are discussed.

A 236-day neutron-induced activity in gadolinium purified by ion-exchange methods is assigned to Gd^{153} . The identification by critical absorption measurements of the europium K x-ray present in the decay of the 236-day activity is the basis of the assignment. The activity is not assigned to Gd^{151} as the Gd^{152} (n,2n) Gd^{151} process with pile neutrons is unlikely. On the basis of coincidence studies a simple decay scheme is proposed in which the Gd^{153} decays to an unstable Eu^{153} by K electron capture and then to the ground state by emission of a 102 Kev γ -ray. Coincidence measurements established the fact that only one γ -ray was present in the decay of Gd^{153} . No electrons with energy greater than conversion electrons of the 102 Kev γ -ray are present.

¹ Doctoral thesis number 1061, submitted June 2, 1950.

The γ -ray is highly internally converted giving rise to K, L, and possibly M electrons.

Activities with half-lives of 7.2 ± 0.2 day and 18- to 24-hour present in the neutron irradiated gadolinium were separated on an ion exchange column. As the 7.2-day activity which preceded the short-lived activity off the column did not grow back into the latter activity, a parent-daughter relationship does not exist with the two nuclides. The 7.2-day activity assigned to Tb^{161} decays by emission of 0.50 Mev β and a 0.05 Mev γ . The 18- to 24-hour activity tentatively assigned to Gd^{159} decays by emission of 0.82 Mev β and γ -rays of 0.05 and 0.42 Mev.

Half-life measurements on gadolinium produced by deuteron bombardment of europium extend over 600 days. The activity decaying with a half-life of 155 days initially is slowly lengthening into a longer half-life. A 265 Kev γ -ray is detected in addition to the 102 Kev γ -ray previously found in Gd^{153} . The 265 Kev γ -ray is assigned to Gd^{151} which decays with a half-life of 150 days. This half-life is based on ratios of the 102 Kev γ -ray to the 265 Kev γ -ray observed over a 600-day period. On the basis of coincidence studies, the decay scheme of Gd^{151} appears to be very similar to Gd^{153} with only the 265 Kev γ -ray present in the decay.

Energy measurements on Eu^{152} and Eu^{154} produced by neutron and deuteron bombardment of Eu_2O_3 are quite similar. This indicates that the same ratio of isotopes is produced in the two irradiations. Half-life measurements on the combined samples of Eu^{152} and Eu^{154} extend over 550 days giving values of 6.6 to 8.5 years.

UTILIZATION OF NITROGEN BY THE ANIMAL ORGANISM. V.
INFLUENCE OF CALORIC INTAKE AND METHIONINE
SUPPLEMENTATION ON THE PROTEIN METABOLISM OF
ALBINO RATS FED RATIONS LOW IN NITROGEN AND
CONTAINING VARYING PROPORTIONS OF FAT¹

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In 1946, it was demonstrated in the laboratory of the Foods and Nutrition Department that dietary fat played an important role in preventing the breakdown of body tissue in a nitrogen-starved animal. When the caloric intake was restricted to one-fourth of the normal intake, the quantity of body nitrogen lost was nearly doubled when fat was absent from the diet. Methionine, offered in quantities supplying 4 mg. of nitrogen daily, reduced the breakdown of body tissue to a level characteristic of rats receiving the diet containing 20 per cent fat and limited in calories.

The nature of the protein-sparing actions of dietary fat and methionine has been examined in the present investigation. Male albino rats were fed low nitrogen diets fortified with adequate amounts of mineral salts and vitamins. One of the rations was deficient in fat, except that supplied in two drops of Wesson oil and cod liver oil daily; the other contained 20 per cent fat. The rations were fed *ad libitum* for 14 days, then 56 calories per day per 300 g. rat being supplied. After a 4-day period of adjustment to force-feeding, nitrogen balances were determined for a 5-day period.

At the end of 23 days, one-half of the rats receiving each diet was divided into four groups and force-fed either (1) the same diets at the same caloric intake as had been administered previously, or (2) the same diets at 25 per cent of this caloric intake. The remaining animals were divided into four groups and placed on similar experimental regimes; in this instance, however, 44 mg. of methionine was added daily to the high and low fat diets offered at the two planes of energy intake. After a 4-day period of adjustment to these procedures, nitrogen balances were again determined over a 5-day period. The animals were then sacrificed, samples of blood were taken and the liver removed. Samples of urine collected in the balance test were prepared for chromatography of urine amino acids and nitrogen partition of urine. The blood was analyzed for the respective concentrations of urea, amino

¹ Doctoral thesis number 1075, submitted June 6, 1950.

nitrogen, and alkaline phosphatase; the liver for moisture, fat, nitrogen, riboflavin, niacin, and glycogen. The weights of livers and adrenal glands were determined and the histology of the liver studied. Comparable groups of animals were used for the determination of glucose tolerance. These data were compared with similar observations made on normal rats fed the stock colony ration.

Space does not permit a discussion of all experimental findings. Only the most important of these will be described.

The marked body sparing actions of fat and methionine were confirmed in these studies using the force-feeding technique. When full calories were fed, the supplementation of the high fat-protein free ration with methionine reduced the negativity of the nitrogen balances from 288 to 215 mg. When calories were restricted, catabolism was increased in both groups, body tissue being used as a source of energy. The surprising observation, however, was the tremendous output of nitrogen in the urines of rats given the low fat diet. The supplementation of the ration of these animals with methionine decreased the quantity of urinary nitrogen excreted, nitrogen balances changing from -1465 to -495 mg. over the 5-day period. The latter balance approximated that of the rats given the low-calorie diets containing fat.

The partition of urinary nitrogen when fat was omitted from the ration revealed that the increased excretion of nitrogen observed when caloric intakes were restricted could be accounted for by increases in the quantities of urea and ammonia, that of ammonia being disproportionately large. Methionine-supplementation of the low calorie-low fat ration returned the excretion of total urinary nitrogen to a quantity characteristically eliminated by rats fed the high fat diet in restricted quantities. However, the proportion of ammonia to urea remained abnormal.

It is interesting to note that chromatographic analyses revealed the presence of all amino acids in the urine, except methionine. In the case of rats receiving inadequate calories, the quantities of aspartic acid, glutamic acid, and glutamine were high.

The concentration of amino nitrogen in the blood was high in both experimental groups receiving the unsupplemented diets at full caloric intake; supplementation of these rations with methionine induced a striking reduction in this constituent. When the caloric intakes of the animals receiving the high and low fat diets were restricted to one-fourth of the normal ingestion, however, blood amino nitrogen values were high for the animals fed the high fat ration and essentially normal for the group fed the low fat ration. The supplementation of the high fat ration with methionine reduced the blood amino nitrogen concentration slightly.

The concentration of blood urea was low when the two test diets were fed at full caloric intake. When the low fat ration was restricted as to calories, however, an increase in the quantity of this constituent in the blood was observed. Here again, methionine was effective in bring-

ing the level of urea to normal, reflecting undoubtedly the concomitant reduction in the quantity of total nitrogen excreted in the urine.

In general, the data relating to nitrogen metabolism suggest that a break occurs in the urea-producing mechanisms when a protein-free diet is fed, a disturbance that is accentuated by removal of dietary fat. The high excretion of ammonia by the rats fed the restricted low fat diet may reflect a necessary metabolic by-path, since the normal animals excreting equally large quantities of nitrogen seem to be able to effect the synthesis of urea without difficulty.

The somewhat distorted glucose tolerance curve characteristic of the animals fed the synthetic protein-free diet containing fat at an adequate caloric intake became more diabetic-like in character when fat was omitted from the ration. All curves became more like that of the normal animal when methionine was added to the rations, both when full and restricted calories were administered. Whether or not additional dietary thiamin would change this picture, needs investigation.

When the protein-free rations were fed, decreased quantities of riboflavin were found in the liver. Omission of fat from the ration resulted in a further alteration of the concentration of this vitamin, it being reduced by 75 per cent in animals fed full calories. Supplementary dietary methionine brought the quantity of this liver constituent to a level characteristic of animals which received fat in the diet. Likewise, the concentration of niacin fell in the livers of rats fed both test diets at full caloric intake; greatest losses, however, occurred in rats receiving the low fat rations; here again, methionine exerted a beneficial effect. Ratios of each vitamin to hepatic nitrogen indicated that the decrement in the quantity of each vitamin, except in one instance, paralleled losses in hepatic nitrogen. The exceptions occurred when the low fat rations were fed; in the case of riboflavin at full caloric intake, in the case of niacin when the low fat diet was administered at restricted caloric intake. It seems possible, therefore, that dietary fat defers the disintegration of some important protein-containing enzyme systems in the liver when the protein-free rations are fed.

This concept is supported by histological studies of hepatic tissue. Methionine was responsible for a more normal cellular structure, an improvement in the quality of the cell membrane, and a more even distribution of cellular components. Also, when caloric intakes were restricted, the presence of faintly staining areas in hepatic tissues suggested that the cytoplasm was characterized by an abnormal type of protein. These areas which were common to both the normal animals fed restricted intakes and those fed the high and low fat rations disappeared in large measure when methionine was added.

Whether the effect of methionine is mediated through maintenance of the status quo of certain cells in organs like the liver, through provision of building blocks for the elaboration of enzyme systems lost in the depletion processes imposed, or through some function it has over and above its role in the synthesis of tissue, it is difficult to state.

That there too is a vital need for dietary fat, over and above the provision of calories and unsaturated fatty acids, has been indicated by abnormalities in metabolism and nutritional well-being when fat is omitted from the diet. The marked distortion in concentrations of specific body components and metabolites when fat is omitted from the ration supports this hypothesis. That methionine and fat exert similar influences on certain phases of protein metabolism is suggested by the nature of changes induced when each supplements the low fat ration.

ERYSIPHE GRAMINIS IN BARLEY. I. EFFECT OF
TIME OF INFECTION AND METHOD OF PLANTING
ON DEGREE OF INFESTATION;
II. MODE OF INHERITANCE OF RESISTANCE¹

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One resistant and four susceptible varieties of barley were inoculated with *Erysiphe graminis hordei* (DC) Marchal at seedling, late tillering, jointing, flowering, and milk stages of plant development under field conditions at Guelph, Ontario, Canada during 1948. Sulfur spray was used to prevent natural spread of mildew until the appropriate time for inoculation.

Mean reductions in yield of the inoculated susceptible varieties when compared with sulfur-sprayed checks ranged from 39.2 per cent when infection occurred in the seedling stage to 3.5 per cent when infection occurred during the milk stage. Reduction in yield was greatest in the varieties which were most heavily infested by mildew. Yield reduction was effected mainly through a decrease in the number of kernels per spike. Differences in kernel size due to time of infection by mildew were small although significant at the 1 per cent level. Kernel size was reduced approximately 7 per cent in the two most susceptible varieties when inoculated in the seedling or late tillering stage.

Mildew had no measurable effect on tillering, sterile spikelets, heading date, maturity date, plant height, or protein content.

Frequent applications of sulfur spray at rates from 5 to 15 pounds per acre depending on plant size were found to be effective in preventing powdery mildew. No effect of the spray was noted on any of the characters measured in the resistant variety.

Mildew determinations at heading time under field conditions on 225 F₃ and parent lines of four crosses sown solid, in 4-inch and in 8-inch spacings were found not to differ significantly although mildew infestation was greatest in solid planting and least in 8-inch spacing in 107 out of the 189 lines which proved to be susceptible. The mean mildew infection in solid, 4-inch and 8-inch spacing was found to be 34.5, 27.2, and 25.3 per cent respectively. Correlations of +.68 and +.72 were obtained when mildew determinations in the 4-inch and 8-inch spacings were compared with mildew infection in solid planting.

¹ Doctoral thesis number 1039, submitted March 13, 1950.

Crosses of the resistant varieties Psaknon, Stephan, Duplex x Atlas Sel. 175, and Kwan, with susceptible varieties indicated that a single dominant gene was involved in each case when F_2 and F_3 progenies were inoculated with race 9 of *E. graminis hordei*. The variety Duplex was found to possess two dominant genes for resistance which were linked with a crossover percentage of 35.82. The gene for resistance to race 9 in Stephan was found to differ from those in Duplex, Psaknon, Selection 175, and Kwan.

USE OF HEAT EXCHANGERS IN THE VENTILATION OF ANIMAL SHELTERS¹

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The ventilation of animal shelters has been a continuing problem for many years resulting in considerable research and many publications. Much effort has been directed toward a solution which would be economically feasible as well as provide satisfactory results. Writers now seem in agreement that ventilation should not only improve the purity of the shelter air and eliminate odors but, perhaps primarily, remove moisture and prevent condensation which may have detrimental effect upon the structural elements. In a poultry house, there is the further problem of keeping the litter dry thus avoiding frequent change and reducing the cost of litter, the labor of changing it and the cleaning of eggs. It has also been observed that old droppings in the litter contain a valuable animal protein factor which has a favorable effect upon the growth and livability of chickens. Keeping the litter sufficiently dry to remain in the house a longer period will add therefore a vital health factor.

The only source of heat in the shelter is that produced by the animal. In addition to offsetting heat losses through walls and ceiling, the animal heat should be sufficient to vaporize water produced within the shelter and heat the circulating air necessary to absorb and remove the moisture.

It is necessary therefore to evaluate the heat and moisture production and also that portion of the moisture which must be removed by the circulating air.

The heat and moisture production of the cow may be obtained from the works of Armsby, Kriss, Forbes, Mitchell, and Hamilton. More nearly accurate results are expected from the recent unpublished work done by Professor Brody in his Animal Psychroenergetic Laboratory in Columbia, Missouri.

The heat and moisture production of the hen and the moisture to be removed from the pen have been calculated from the data obtained from Mitchell and Kelley, Barott and Pringle, Oliver and White research work.

A study of the heat balance for certain animal shelters shows that there is a deficiency and that the heat produced is not enough to cover all the losses. Figure 1 shows a typical heat balance for a dairy barn. Part A of the heat produced is used for vaporization of moisture. Part

¹ Doctoral thesis number 1027, submitted February 6, 1957.

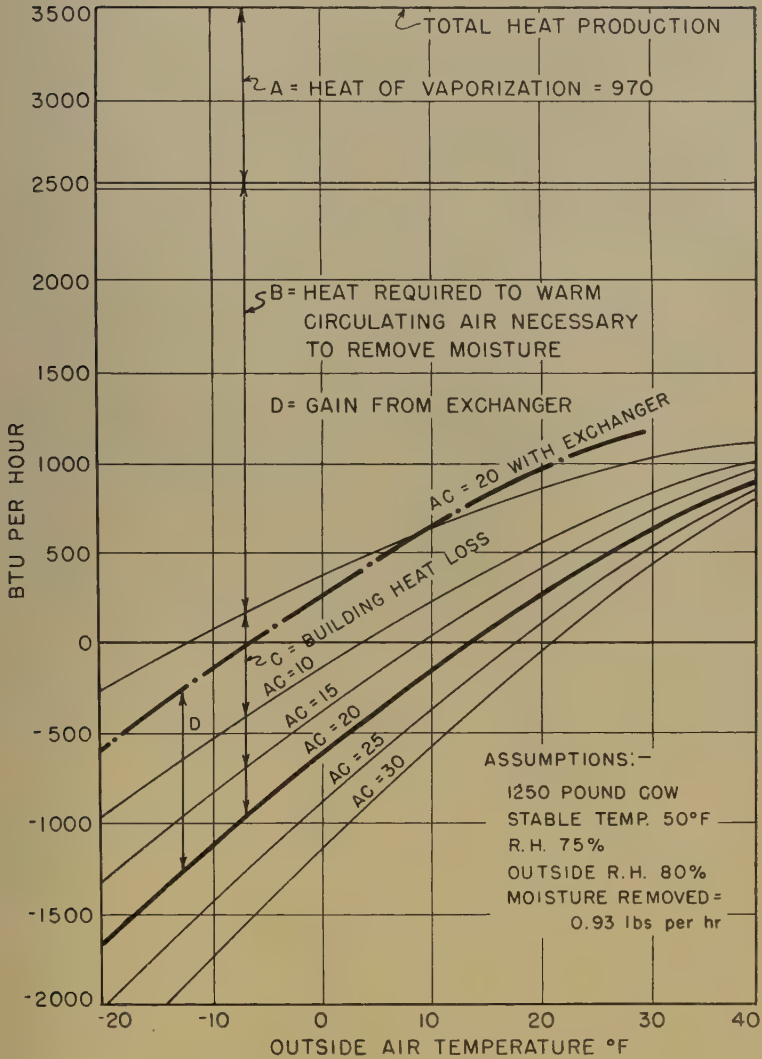


Fig. 1. Heat balance in a dairy barn.

B is used in warming circulating air necessary for ventilation. Part C is the heat loss through the building. This last item depends upon the construction of the shelter.

It is therefore necessary either to restrict the ventilation or to add

a device by which we will add heat or facilitate moisture removal. We may have four alternatives to this device:

1. Direct heating by furnace or electric coils.
2. A heat pump which can be used for heating as well as cooling.
3. A heat exchanger.
4. Chemical absorbents.

In all these alternatives, except the heat exchanger, the heat energy gained or the chemicals must be paid for besides the cost of the equipment necessary for ventilation. In the heat exchanger, the heat energy will be obtained by depleting the exhaust air of its energy through the communication by a metal sheet wall between this air and the incoming fresh air. In other words the heat exchanger will recover a certain per cent of part *B* in Fig. 1.

The heat exchangers used in our experiments in the College Poultry and Dairy Farms were built of small pipes inside ducts. The exhaust air moved in the small pipes while the fresh air flowed in the annular space. The flow of the exhaust was either parallel or counter to the flow of the fresh air. In the finally suggested heat exchanger, the flow is a combination of parallel and counter flow. This type of flow is called reverse flow. The system is formed of small pipes inside a duct which is encircled by a second duct. This second duct is used as the inlet of the exhaust air.

Heat gain by the exchanger:

Coefficients of heat transfer can be obtained by established formulae concerning conduction, convection, and radiation. A solution can be made to any heat exchanger and curves of performance can be drawn for different conditions of operations. An equation which presents the heat gain can be put in the form

$$H = k_1(t_i - t_o) + k_2(t_i - t_o)V$$

where t_i and t_o are temperatures of the inside and outside air and V is the volume of air passing in the system. k_1 and k_2 are constants obtained from the curves.

Highly satisfactory results were obtained when heat exchangers were installed in some animal shelters. These results were clear and obvious when better and similar structures were compared to those which were equipped with the exchanger.

In the theoretical study of the heat balance of the barn, a glance at $AC = 20$ will show clearly that the inside temperature of the barn cannot be maintained at 50°F . with the ordinary methods of ventilation if the outside temperature is below 14°F . If this same barn is equipped with a heat exchanger, the temperature can be kept at 50°F . even if the outside temperature drops to -6°F . Comparable benefits are also attainable in a poultry house.

It is a fact, therefore, that if any normal ventilating system succeeds in solving some of the requirements, the heat exchanger will add more success since the heat gained by warming the fresh air will help in keeping the temperature level inside the shelter higher than if we do not have this equipment.

The choice of heat exchanger involves the consideration of a number of factors, some of which are:

1. The larger the surface area for heat transfer the more heat is gained. This result can be obtained by having several small pipes. For the same cross sectional area, the small pipes have a larger surface area than that of bigger pipes.

2. The higher the velocity of the fluid, the higher is the gain.

It should also be put into consideration that the larger the surface area and the higher the velocity, the greater is the power needed to push the air into the system.

3. The larger the mean temperature difference the greater is the transfer of heat.

4. The greater the amount of condensation, the greater is the over-all coefficient of heat transfer and consequently the gain. Condensation will occur when the warm humid exhaust air touches the cold wall separating it from the cold fresh air. To increase this condensation, we have to increase this surface area of contact. Also moving humid air will condense more than still humid air. The suggested double duct heat exchanger fulfills these conditions.

RECOVERY OF SOLVENT FROM SOYBEAN OIL-SOLVENT SOLUTIONS BY STRIPPING IN PACKED COLUMNS¹

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Tests with various packing materials showed that the stripping performance of a packed column is determined by the following factors: (1) temperature and pressure, (2) column dimensions, (3) packing characteristics, and (4) liquid distribution. Prior to this research, very little consideration had been given to the effect of liquid distribution on the efficiency of packed columns. As a result of these studies, methods of controlling liquid distribution were developed and applied to the more efficient utilization of packing materials as stripping media.

A rotating twenty-point distributor head was devised for obtaining uniform liquid distribution in a tower and a packing unit was developed which maintained the initial distribution pattern nearly perfectly throughout the packed height of a column. The packing unit was composed of spiral weave metallic cloth rolled into units spaced with ½-inch Berl saddles. It was found that the metallic cloth possessed filming properties which made it far superior as a packing material to either dumped 1-inch Berl saddles or 1-inch Raschig rings.

Column performance obtained with metallic cloth packing units was evaluated by determining the stripping efficiency and limiting capacity of the column. Thus, by controlling liquid distribution within a packed column, a very compact and highly efficient packing unit was developed which may be applied directly to commercial stripping operations in the soybean oil industry. This unit has approximately five times the stripping efficiency and three times the capacity of an equal quantity of dumped 1-inch Berl saddles or 1-inch Raschig rings. Tests showed that reinforced metallic cloth was superior to the non-reinforced type.

Rolled units composed of galvanized wire screen and ½-inch Berl saddle spacing were investigated, but they gave very poor results when employed as stripping media. Fiberglass packing was subjected to both distribution and stripping tests. In each case, it gave very poor results, and this was easily understood when observations revealed the marked tendency of the material to displace large quantities of liquid toward the column wall.

The effect of stripping contact time on soybean oil color at relatively high temperatures was investigated. Results showed that the relation

¹ Doctoral thesis number 977, submitted July 5, 1949.

between these two variables could be expressed mathematically by the general equation:

$$C = k e^{mt}$$

where C is the lovibond, red, color and t the contact time. The term k is a constant determined by the initial oil color and m is a constant determined by actual stripping conditions.

MINERALIZATION OF INOSITOL-BOUND PHOSPHORUS IN SOIL¹

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Previous investigations indicate that inositol phosphates comprise a large portion of the organic phosphorus in soils. The present investigation was conducted to obtain information on the behavior of inositol phosphates that would contribute to an understanding of the importance of these phosphates in plant nutrition.

Laboratory studies were carried out on the solubility and phytase-catalyzed hydrolysis of the iron, aluminum, calcium, and magnesium salts of phytic acid and phytic acid derivatives. This work was followed by studies on the source of phytase in soil, the relationship between the phytase activity and the over-all microbiological activity in soils, and the effect of the variation of some soil properties on the level of phytase activity. Finally, experiments were conducted to determine the relative importance of phytate solubility and phytase activity in controlling the rate of mineralization of soil phytates. In the course of this work methods were developed for preparing relatively pure sodium phytate, for measuring the phytase activity in soils, and for determining the phytate phosphorus content of soils.

A solution of sodium phytate was prepared from commercial calcium phytate. The original, impure, calcium salt was dissolved in 2 per cent hydrochloric acid, and the extraneous matter was filtered off. The calcium salt was reprecipitated with sodium hydroxide, filtered, and then redissolved in 8 per cent acetic acid, from which it was reprecipitated in relatively pure form by boiling. This precipitate was dissolved in dilute hydrochloric acid, ferric chloride was added, and the resultant precipitate of ferric phytate was filtered off and thoroughly washed with 2 per cent hydrochloric acid. This washing removed the lower derivatives of phytic acid, leaving essentially pure ferric phytate. The ferric phytate was decomposed with sodium hydroxide, the ferric hydroxide was filtered off, and the copper salt was precipitated from the resulting sodium phytate solution with excess copper acetate. In turn the copper salt was decomposed with hydrogen sulfide, the excess hydrogen sulfide was removed by aspiration, and the resultant solution of phytic acid was titrated with sodium hydroxide to pH 8. This solution was used in the solubility studies after suitable dilution.

A solution of sodium phytate derivatives was prepared from a sodium phytate solution by hydrolyzing off half the phosphorus with

¹ Doctoral thesis number 1004, submitted October 15, 1949.

a phytase-active extract of bran. The solution was then decolorized with charcoal, and the calcium salt was precipitated. This precipitate was dissolved in dilute nitric acid, and the major part of the inorganic phosphorus was precipitated as the ammonium molybdophosphate while the last fraction was removed by isobutyl alcohol extraction in the presence of ammonium molybdate. The calcium salt was reprecipitated, dissolved in acetic acid, and the lead salt was then precipitated with lead acetate. This lead salt was then decomposed with hydrogen sulfide, and after the excess hydrogen sulfide had been removed by aspiration the acid solution was titrated to pH 8 with sodium hydroxide. This solution was suitably diluted and used in the solubility studies. It had 2.7 atoms of phosphorus per molecule of phytate derivative.

In the solubility experiments the sodium phytate and phytate derivative solutions were mixed with solutions of the chlorides of the cations under consideration in proportions such that the cation to phosphorus equivalent ratios were 1, 3.75, and 6.25. The concentrations of phosphorus in the final mixtures were 50 and 22.5 p.p.m. for the phytate and phytate derivative forms, respectively. The pH was adjusted with dilute acid or alkali, and after the mixtures had stood for 3 to 11 days they were filtered through sintered glass crucibles supporting a layer of acid-washed, ball-milled quartz sand. The filtrates were then analyzed for phosphorus. The results showed three main features: first, that the iron and aluminum salts were very insoluble under acid conditions, whereas the calcium and magnesium salts were fully soluble at reactions less than pH 5 and pH 6.5, respectively; second, that the phytate derivatives were more soluble than were the phytates especially in the case of the calcium and magnesium salts; and third, that the presence of excess cations decreased the solubilities in the case of the calcium and magnesium salts, but with iron and aluminum either caused little change or else increased the solubility at low pH values. This increase in solubility was ascribed to the formation of relatively stable supersaturated solutions and/or the formation of soluble complexes. For the phytates and with the cations in equivalent and excess amounts, respectively, the pH ranges of essentially full insolubility were as follows: for iron from pH 1 to pH 3.5 and from pH 2.5 to pH 8; for aluminum from pH 3 to pH 4 and from pH 3 to pH 9; and for calcium from pH 8 to pH 9 and from pH 6.5 to over pH 10. The magnesium phytate was fully insoluble only with excess magnesium present and at pH values greater than 10. The calcium and magnesium phytate derivatives were always appreciably soluble, while the iron and aluminum phytates showed respective minimum solubilities at pH 2.5 (8 per cent soluble) and pH 4.5 (20 per cent soluble) when equivalent amounts of the cations were present. With excess iron and aluminum present the derivatives were insoluble over the pH ranges from pH 3 to pH 6 and pH 4.5 to pH 8, respectively.

The results for the phytates were compared to those of the corresponding inorganic phosphates found under very similar conditions

by a previous worker. The corresponding curves were very similar, the principal contrast being that whereas with equivalent amounts of cations present the inorganic phosphorus was always soluble to an appreciable extent, this degree of solubility only applied to the magnesium salt among the phytates. It was concluded that the relatively low solubility of the iron, aluminum, and calcium phytates would explain the small availability of phytate phosphorus to plants. It was further concluded that insofar as this insolubility limits the availability and mineralization of phytate phosphorus, agronomic practices designed to maintain the soil reaction in the range pH 6 to pH 6.5 would be desirable.

In the hydrolysis studies, solutions of the chlorides of the cations mentioned above were added to known amounts of sodium phytate and sodium phytate derivatives. The concentrations of phosphorus in the two systems were 46.3 and 27.4 p.p.m., respectively, and the cations were present in excess except in the case of ferric iron where the cation was present in both equivalent and excess amounts. The reactions were suitably adjusted, and a constant amount of a phytase-active extract of bran was added. The mixtures were then incubated at 45°C. for 1.75 hours, at the end of which time the increase in inorganic phosphorus was determined. Among the iron and aluminum systems only the ferric phytate derivatives series with the iron present in equivalent amount showed definite hydrolysis. The calcium and magnesium systems were all appreciably hydrolyzed especially in the case of the derivatives. In general the hydrolysis curves followed the solubility curves although there was evidence that as the pH values departed from the optimum for the enzyme (about pH 5.2 to 5.8) the activity of the enzyme was reduced and became a limiting factor with the more soluble salts. It was concluded that in long-term hydrolysis experiments and with the exception of those cases where the pH was either very high or low, the main factor limiting the rate of hydrolysis was the degree of solubility of the phytate.

Prior to investigating the phytase activity of soil it was necessary to develop a method of measuring the phytase activity in soil. An empirical method was developed that consisted of incubating 5 g. of the soil for 20 hours at 45°C. with 20 ml. of solution containing 1 g. of potassium citrate and 60 mg. of phytate phosphorus, added as a solution of sodium phytate, and adjusted to pH 5. Toluene was added to prevent microbial activity. At the end of incubation the soil was filtered and washed with 150 ml. of 4 *N* hydrochloric acid. The increase in the inorganic phosphorus in this filtrate over that in the filtrate of a sample similarly treated but with the phytate solution replaced by water was taken as a measure of the phytase activity of the soil.

The effects of storage and drying of the soil prior to determining the phytase activity were investigated. It was found that drying greatly reduced the measured activity in three soils of different texture. The decrease in phytase activity was apparently due to the greater adsorption of the phytase by the soil as the moisture content was reduced.

One soil, previously incubated with sugar, and which had been dried to varying degrees, was stored in closed bottles and the phytase activity was determined after increasing time intervals. At all moisture levels the activity first increased and then slowly declined. The change was undoubtedly due to microbial synthesis of new enzyme followed by slow decomposition of the enzyme. These results showed that the method was largely dependent on the pretreatment of the samples and accordingly was of most value where the samples were comparable as regards their immediately previous history.

The relationship between over-all microbial activity and phytase activity was investigated by measuring the carbon dioxide produced and the phytase activity developed by sterile mixtures of soil and increasing amounts of alfalfa meal when incubated after inoculation from a fresh soil. It was found that the carbon dioxide produced and the level of phytase activity were linearly related, indicating that the higher the microbial activity the higher was the phytase activity in the soil. The results of similar studies using pure bacterial and fungal inocula indicated that phytase production is common to many soil microorganisms. There was some evidence that fungi might be generally high phytase producers. This was supported by the finding that when soils of different pH values were incubated with energy material, the level of the resulting phytase activity was inversely related to the soil pH. It was concluded that phytase activity is probably present in all field soils and that the level of activity may be greater under more acid conditions.

The findings reported so far indicate that the low solubility of the phytates in acid soils rather than the lack of phytase activity is probably the factor limiting the availability of phytate phosphorus to plants. To obtain more evidence on this point two series of mineralization studies were carried out. In the first series, the mineralization of sodium phytate added to soils of different pH values was determined, and in the second series the mineralization of the native phytate phosphorus in soils of different pH values was determined. The results of the first series of experiments showed that the amount of natural phytase in the soil was sufficient to mineralize a relatively large amount of phytate phosphorus when soluble phytate was present in large amounts.

In the second series of experiments, soils high in organic phosphorus were adjusted to pH values of 5, 6, and 7 and incubated for 15 weeks dry, moist, and moist with starch, sugar, and nitrogen added. The soils were then analyzed for phytate phosphorus according to the method described below. Where the soils had been incubated with only water added, there was no decrease in phytate phosphorus irrespective of the soil pH. With the energy material and nitrogen added there was no decrease in phytate phosphorus at pH 5, but appreciable decreases occurred at pH 6 and 7, the greater decrease being found at pH 7. These results may be explained on the basis that at pH 5 the factor limiting mineralization was the low solubility of the soil phytates,

whereas at pH 6 and 7 both phytate solubility and phytase activity were limiting factors. However, preliminary evidence was obtained to indicate that soil microorganisms can synthesize phytates. If further work upholds the present evidence, the explanation of the results obtained in the foregoing experiment must take into account a balance between microbial synthesis and destruction of phytates.

The method developed for the determination of phytate phosphorus in soil was essentially as follows. The organic matter of an 0.5 N sodium hydroxide extract of soil was largely removed by acid precipitation at pH 0.1. The phytate was then precipitated with iron at pH 1.7 to pH 1.8, and the organic matter accompanying this precipitate was destroyed by hypobromite oxidation. This stage was followed by a calcium precipitation under alkaline conditions. The calcium salt was redissolved in dilute acid and the iron precipitation was repeated. Finally, the organic phosphorus in the iron precipitate was determined. The recovery of added phytate phosphorus was complete, and it was estimated that for phytate derivatives it was about 80 to 90 per cent. The recovery of added nucleic acids and other organic phosphates was checked and in all cases was found to be negligible. It was considered that the method is basically sound although still subject to some errors.

In general, the results reported above indicate that soil phytates are relatively stable, especially in acid soils. Soil phytate phosphorus undergoes mineralization, but the extent of mineralization is appreciable only at higher pH values and at high levels of microbial activity. It is probable that under field conditions, where the level of microbial activity is relatively low, phytates supply very little phosphorus to plants during any one growing season.

DEVELOPMENT OF TYROSINE-INDEPENDENT STRAINS OF *LACTOBACILLUS ARABINOSUS*, AND SOME PHYSIOLOGICAL PROPERTIES OF NUTRITIONAL VARIANTS¹

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Lactobacillus arabinosus normally requires tyrosine as a component of its culture medium. However, a culture of the organism can become adapted to a medium containing no tyrosine. In estimations of the frequency distribution of adaptation of cultures to tyrosine-deficient media it was found that there was a significant variation between independent tests in the proportions of cultures which became adapted. The adaptive process was further characterized by an excessively long interval between inoculation and first appearance of adapted cultures.

Tests showed that the variation between tests could not be attributed to differences between inocula. A cause of the variation was found in the fact that batches of tyrosine-deficient media differed in the concentration of normal cells which they could support. Since the probability of adaptation within the first 5 days following inoculation was correlated with the number of cells present, the growth-promoting capacity of the deficient medium was an important factor in adaptation.

Factors which appreciably increased growth-promoting abilities of tyrosine-deficient medium for normal cells were increase in length of autoclaving, increase in concentration of phenylalanine, and to a lesser extent, increase in concentration of histidine and decrease in initial hydrogen ion concentration. Factors which did not significantly affect concentration of normal cells were amount of inoculum, concentration of individual growth factors, and concentration of individual amino acids other than phenylalanine and histidine.

Two reasons for the delay in adaptation of cultures to absence of tyrosine were found to be a general retardation in growth rate of adapted cells in older cultures containing normal cells in tyrosine-deficient medium, and an early advent and later relaxation of an inhibition of adapted cells in the cultures in which they arose.

An hypothesis to account for the renewed growth of the inhibited cells involved a mutation of already adapted cells to resistance to the inhibition. The validity of the hypothesis was based primarily on the following facts. The presence of adapted cells among the normal cells in a deficient medium did not inevitably result in culture adaptation. Subculturing in deficient media was more likely to result in production

¹Doctoral thesis number 1009, submitted December 8, 1949.

of an adapted culture than continuous culturing of normal cells in a single tube of deficient medium.

A culture in the process of adaptation contained at least two types of adapted cells as determined by colony size on plating. These sub-strains were not necessarily stable since on culturing they could become uniform with respect to rate of growth. Cells of an adapted strain multiplied more quickly within cultures of normal cells in deficient medium than did the adapted cells already present in such cultures.

Adaptation of normal cells to tyrosine-phenylalanine-deficient media or to phenylalanine-deficient media could not be obtained. However, adaptation of tyrosine-independent cells to tyrosine-phenylalanine-deficient media occurred with a high frequency.

Properties of tyrosine-independent strains were determined. It was found that strains isolated from different cultures could differ from each other in rate of growth. A strain had complete stability in yeast-extract medium, and a strain in yeast-extract medium had a rate of growth similar to that of a normal strain and was not at a disadvantage in the presence of normal cells.

A study of the ability of normal cells to replace phenylalanine with tyrosine and tyrosine with phenylalanine revealed that tyrosine could freely replace phenylalanine at high or low concentration, and that phenylalanine could replace tyrosine, although for most efficient substitution the concentration of phenylalanine had to be in excess of 200 mg. per liter. Replacement of either amino acid by the other was inefficient in that, for an equivalent extent of growth, the required amount of the single acid far exceeded the total amount of the two acids when both were present.

A study of the effect of temperatures of 31°, 33°, 35°, and 37°C. on rate of growth and extent of growth at 36 hours of a normal, a tyrosine-independent, and a tyrosine-phenylalanine-independent strain in complete synthetic, tyrosine-deficient, phenylalanine-deficient, and tyrosine-phenylalanine-deficient media yielded the principal information listed below. In deficient media, rate of growth of normal cells was greatest at 31°C., both tyrosine and phenylalanine being stimulatory rather than essential at lower temperatures. Rates of growth of the adapted strains in these media were greatest at temperatures above 31°C. In complete medium, a rise in temperature resulted in an increase in rate of growth for all strains. Tyrosine remained stimulatory at all temperatures for the tyrosine-independent strain. Growth rate of the tyrosine-independent strain was higher than that of the normal in phenylalanine-deficient medium. Rate of growth of the tyrosine-phenylalanine-independent strain was lower than that of the normal or tyrosine-independent strain at all temperatures in complete medium. It was lower than that of the tyrosine-independent strain at higher temperatures in tyrosine-deficient medium. For all strains in complete medium, there was a strong tendency for increase in rate of growth to be correlated with a decrease in extent of growth. Extent of growth

in complete medium at 31°C. was greatest for the normal strain, least for the tyrosine-phenylalanine-independent strain. At 37°C. this order was reversed. The temperature of greatest extent of growth of the normal strain was 31°C. in all media. The temperature of greatest extent of growth of the tyrosine-independent strain was 31°C. in all media but tyrosine-deficient, where the temperature was 35°C. The greatest extent of growth of the tyrosine-phenylalanine-independent strain was achieved at 35°C. in all media but tyrosine-phenylalanine-deficient, where maxima occurred at 31°C. and at 35°C.

TISSUE TESTS AS INDICATORS OF MINERAL NUTRITION OF COTTON¹

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Tissue tests have been applied to corn and various truck crops and have afforded a means of estimating the nutritional status of the plant. The application of suitable tissue tests to cotton would be of similar value. The first phase of this program was the selection of the proper chemical methods and the determination of the portion of the plant to be used.

Cotton plants were grown in 3-gallon glazed pots filled with washed creek sand. The plants were divided into four groups corresponding to the nutrient treatments employed.

On each of the following dates—August 1, 8, 15, and 22—a typical plant was harvested from the base treatment and divided into its component parts. Soluble NO_3 nitrogen, phosphorus, and potassium were determined by colorimetric and turbidimetric means using a Klett Summerson colorimeter. The results of these tests indicate that NO_3 nitrogen increased in concentration as the main-stem apex was approached. The main-stem leaf blades were low in NO_3 nitrogen, whereas the main-stem petioles were consistently high in that constituent at all four dates.

Little difference was noted in the concentration of soluble phosphorus between the various plant fractions. The main-stem leaf blades were higher in that element than the other portions of the plant.

Potassium tends to accumulate in the petioles, as was evident from the high concentration of soluble potassium in the petioles harvested August 1. It was found that petiole nitrogen and potassium reflected their respective concentrations in the substrate. Phosphorus in the petioles was not correlated with its supply. A significant negative interaction was obtained between substrate nitrogen and petiole phosphorus.

A greenhouse experiment was started in November of 1947 to study the problem of tissue tests of cotton in more detail. Thirteen nutrient treatments were used thus affording a "high," "medium," and "low" treatment for nitrogen, phosphorus, sulfur, potassium, calcium, and magnesium. Harvests were made at 60, 90, and 145 days after emergence. This experiment was repeated in the winter of 1949. A harvest of tops was made at 30 days in addition to the three petiole harvests previously mentioned.

From these two greenhouse experiments it was evident that the

¹ Doctoral thesis number 1065, submitted June 2, 1950.

concentrations in the petioles of the five elements tested for reflected their respective levels in the substrate. In both experiments, significant positive correlations were obtained when petiole nitrogen, phosphorus, potassium, calcium, and magnesium were compared with their respective substrate levels.

From the two greenhouse studies it was evident that indications of nutrient deficiencies can be obtained by tissue tests in advance of the appearance of typical deficiency symptoms. The exact age at which deficiencies could be detected by tissue tests is difficult to establish. In the greenhouse experiment of 1949, deficiencies were noticed as early as 30 days after emergence of the seedlings. The present data indicate that the time would vary with the severity of the deficiency. Under favorable growing conditions it may be possible to determine a deficient supply of nitrogen by tissue tests within 2 or 3 weeks after emergence. On the other hand, a level of some nutrient element which is not sufficient under rapid growing conditions may be ample under environmental conditions not favoring rapid growth.

Interactions were observed between nutrient elements. An inverse relation was found consistently between petiole phosphorus and substrate nitrogen. A low or limiting supply of potassium was reflected by the accumulation of calcium and magnesium in petioles. A significant negative correlation was obtained between substrate calcium and petiole phosphorus. Other interactions appeared sporadically throughout the course of these investigations, but their importance is questionable.

Tissue tests were applied to field grown cotton and the results obtained substantiate those of the greenhouse experiments.

TABLE 1
CRITICAL CONCENTRATIONS OF PETIOLE NITROGEN, PHOSPHORUS, AND POTASSIUM OBSERVED
IN 90-DAY OLD FIELD GROWN AND GREENHOUSE COTTON
(Results based on fresh weight)

	Percentage in Petioles		
	NO ₃ N	P	K
Field Grown.....	.03	.009	0.7
Greenhouse.....	0.10	.019	0.7

It has been suggested that the limiting of growth by a deficiency of some element is the cause of the accumulation of other elements within the plant tissue. Data were obtained during the 1947 greenhouse experiment which did not substantiate this conclusion and made it evident that the question of interactions is not adequately explained by limited growth alone.

Critical levels of nitrogen, phosphorus, and potassium in the petioles, which were observed in both the greenhouse experiments and the field study, are shown in Table 1. Plants containing less than these critical

concentrations in their petioles gave reduced yields. In general, the agreement between the field and greenhouse plants is good.

A wide difference is noted between the nitrogen levels, but it must be remembered that the field grown plants had other supplies of nitrogen than the nitrates shown by the tests. The phosphorus level of the greenhouse plants appears high. The reduction in yield due to phosphorus in the greenhouse experiments was not statistically significant, thus the .019 per cent phosphorus may be somewhat higher than the true critical concentration. The comparison between potassium levels is striking.

During the course of the greenhouse experiments it was observed that the plants receiving the least amount of nitrogen had the highest relative fruitfulness. It was noticed that the high nitrogen plants produced bolls on fruiting branches which developed from the fifth or sixth node from the base of the main stem. In contrast to this, fruiting branches which held bolls first appeared on the eleventh or twelfth node of the low nitrogen series. The high nitrogen plants, which had been vigorously vegetative for the first 60 days, became predominantly fruiting in their activity. The low nitrogen plants remained vegetative over a longer period of time and matured their crop much more slowly but eventually produced more bolls in proportion to total growth.

From these studies it is evident that tissue tests can play an important part in determining the nutritional status of cotton plants. At present, it seems that the interpretive value of information gathered from tissue tests is greatest at the end of the growing season; however, indications of deficiencies by tissue tests were obtained as early as 30 days after emergence.

CYTOPATHOLOGY OF THE HEMOCYTES OF *TENEBRIO MOLITOR* LINNAEUS (COLEOPTERA)¹

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Total hemocyte counts (T.H.C.) and differential hemocyte class counts (modified Yeager system [1945]) from mealworm larvae (*Tenebrio molitor* Linnaeus) have been used, in addition to some special hematological counts, in studying the effects of various physiological and pathological conditions on this insect.

These studies indicate that in mealworm larvae simple hemorrhage, short starvation, desiccation, and parenteral injections of saline, acetone-saline, and distilled water generally do not materially alter the total or differential hemocyte counts. On the other hand, abdominal ligation, cautery, and topical applications of p,p'-DDT, 10 per cent DDT dust, DDT in acetone, and nicotine vapors tend to alter the blood picture in a number of complex ways. Parenteral injections of lamp black, India ink, colloidal gold, colloidal iron, DDT in acetone saline, and olive oil emulsions generally tend to alter the blood picture of *Tenebrio* larvae. Benzene hexachloride (containing 1 per cent *gamma* isomer), toxaphene (5 per cent and 10 per cent dust), and 95 per cent sodium fluoride applied topically to the mealworm did not alter the differential hemocyte class counts.

Total hemocyte counts from larvae reveal that both hemocytoses (counts above 70,000 cells/mm³), comparable to leucocytoses in vertebrates, and hemocytopenias (counts below 20,000 cells/mm³), comparable to leucopenias in vertebrates, occur in some insecticidally poisoned insects. Thus, marked hemocytoses seem to be characteristic for mealworm larvae subjected to nicotine vapors for 48 hours. Hemocytoses appear in the early convulsive stages of DDT poisoning, and in ligatured larvae. Marked hemocytopenias are generally associated with prolonged starvation, in the late stages of acute and chronic poisoning, and in final stages of septicemias.

Pathological hemocytoses in mealworm larvae are frequently associated with pronounced increases in plasmatocytes (phagocytic hemocytes) and by increases in primitive stem cells and transitional hemocytes (prohemocytoids and smooth-contour chromophiles, respectively). Cystocytes or the coarsely granular hemocytes of the mealworm, which together with the plasmatocytes normally make up approximately 96 per cent of the hemocyte classes encountered in the hemolymph of the

¹ Doctoral thesis number 1029, submitted March 6, 1950.

mealworm, tend to be more prevalent after long starvation periods. The function or functions of the cystocyte are not known, but it is suggested that they act trephocytically. In the mealworm the cystocytes are not phagocytic.

The following percentage counts proved useful in studying the effects of various factors on the hemocytes of the mealworm: (1) mitotic cells (1,000 cells counted), (2) phagocytic index (100 cells counted), (3) poikilohemocytes (abnormally shaped hemocytes of all categories), (4) hemocytes with hypertrophied nuclei (hemocytes in all categories), (5) hemocytes with small nuclei (arbitrary limits chosen: 3.5 micra), (6) grossly vacuolated hemocytes of all categories, (7) number of plastids (cytoplasmic fragments), (8) free fat body cells, (9) fusiform cells of all categories, and (10) visibility pathologic hemocytes.

The present studies indicate that when an insecticide affects the number and morphology of mealworm hemocytes, total and differential hemocyte counts may frequently be correlated with symptomatology. Such counts then seem to furnish one index to the internal milieu of the insect.

Since the hemocyte picture of the mealworm is more complex than appears to be the case in a number of other Coleoptera, as well as of some other insect orders, and less complex than in the southern armyworm (*Prodenia eridania* Cram.), it appears unwise to assume that these findings apply to other insects.

The techniques used in this study provide quantitative tools for the analyses of many problems in insect physiology and toxicology.

COMPOSITE SIBBING VERSUS SELFING IN THE DEVELOPMENT OF CORN INBRED LINES¹

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Low yields and poor vigor of inbred parents of hybrid corn have been a source of trouble to plant breeders and seed producers. This is especially true in those areas outside the Corn Belt proper where average yields are low because of low soil fertility and great seasonal hazards. In the southern corn growing areas increase blocks of inbred lines or single crossing fields are often partial or complete failures.

S₁ lines of corn are expected to possess vigor intermediate between the original open-pollinated selection and the homozygous lines which may be isolated from it. This led to speculation as to whether or not it might be possible to utilize the greater yielding ability of less highly inbred lines in the production of commercial hybrids. With this end in mind, lines were established by selective mass sibbing each within a closed population representing an S₁ progeny. These will be referred to as composite-sibbed lines and designated by the symbol C#. C#₂, for example, would indicate a line established by one generation of self-pollination followed by two generations of selective mass sibbing. The S₁ lines to establish these lines were selected for uniformity, vigor, and combining ability. More nearly homozygous lines also were isolated by continuous selfing in the progeny of the same original self-pollinated ear from which a C# line was established.

In order to compare agronomic characteristics and variability, seven C# and S₄ lines derived from the same original S₀ selections were grown in a split plot field experiment with five replications. Individual plant measurements were taken for days to silk, number of tassel branches, plant height, and ear weight. C# lines proved more vigorous than related S₄ as measured by yield, plant height, and earliness. Average yields, uncorrected for stand differences, were 26.0 and 10.2 bushels per acre for C# and S₄ lines respectively.

In order to compare relative variability of C#₃ and S₄ lines, coefficients of variation for the four plant characteristics measured were calculated for each plot. Although consistent differences in variability of C# and S₁ lines could not be demonstrated except in the case of days to silk, C# lines usually had slightly higher coefficients of variation for days to silk, number of tassel branches, and plant height. A reversal of relative variations occurred when ear weight was considered; in most cases the C# lines actually possessed lower coefficients of variation.

¹ Doctoral thesis number 1024, submitted January 16, 1950.

Theoretically the S_1 lines should be essentially homozygous while C# lines should be only slightly more than 50 per cent homozygous. Since vigor measurements, especially yield, bear this out, it would be expected on the basis of genotype alone that C# lines should be nearly twice as variable as S_1 lines. The data did not substantiate this supposition. The effect of environment in modifying expression of genotype was apparently much greater upon the less vigorous S_1 lines than upon the relatively more vigorous C# lines.

In order to compare hybrid combining ability of C#₂ and S_3 lines, test crosses of eight composite-sibbed lines and their related S_3 lines were compared in a split plot field experiment with eight replications. Measurements of individual plants for plant height and yield were taken in four replications to compare variability. No consistent differences in combining ability of C# and S_3 lines derived from the same original selection could be detected. Mean yields of test crosses were 73.9 and 74.3 bushels per acre for C# and S_3 lines respectively. Variability in plant height and yield of test crosses involving C# and S_3 lines were not appreciably different.

Test crosses of 16 S_1 plants, 40 C#₂ plants, and 24 S_3 lines, all of a single line of descent, together with the tester parent, were compared in a yield trial. No appreciable differences among the means of the groups could be detected. Mean yields were 64.2, 63.7 and 63.6 bushels per acre for S_1 , C#₂ and S_3 respectively. Significant segregation for combining ability occurred among the S_3 lines, but no S_3 test cross was significantly higher yielding than the mean of the C# test crosses. This gave an indication of the difficulty of isolating homozygous lines superior in combining ability to the original selection from which they were derived.

Certain precautions need to be observed if composite-sibbed lines are used as parents of corn hybrids. Since the characteristics of lines maintained by this technique will never be as completely fixed as homozygous lines, constant care will be required to eliminate off-type plants and preserve uniformity. In each generation of maintenance sufficient male and female plants need to be used as parents to minimize inbreeding. When either C# lines or their single crosses are used in making experimental hybrids, sufficient plants must be used in order to obtain an adequate sample of their germplasm. It is necessary that those working with C# lines become more thoroughly acquainted with their material than when homozygous inbred lines are used.

Composite-sibbed lines are suggested to replace homozygous inbred lines as parents of corn hybrids in areas where maintenance of inbred lines is difficult. Carefully selected C# lines should be more vigorous and not sufficiently more variable than more highly inbred lines to prohibit their use in production of hybrid seed corn. At least no great loss in yielding ability of hybrids would be expected when composite-sibbed lines are used. In a newly initiated corn breeding program composite-sibbed lines might well be used as a stop-gap in production of

adapted hybrids before desirable pure lines can be developed and tested. Composite-sibbed lines are ready for final testing immediately without the additional generations of selfing required for homozygous lines. In areas where homozygous lines can be maintained in a satisfactory manner they would probably be preferred because of the more exact genetic control possible in their hybrids.

HOST-PARASITE RELATIONSHIP BETWEEN *CYNAEUS* *ANGUSTUS* LEC. AND *NOSEMA CYNAEA* SP. NOV.¹

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From 1940 to 1942 the author studied the biology of a pest of stored corn, *Cynaesus angustus* Lec., family Tenebrionidae, order Coleoptera. A protozoan parasite of the insect, identified by Dr. E. R. Becker of Iowa State College as a microsporidian, was discovered in 1942.

A study of the host-parasite relationship of the microsporidian and its host was undertaken in 1946. The amoebula, meront, sporont, sporoblast, and spore stages in the development of the parasite were observed and are described. The parasite was determined to be a new species and the name *Nosema cynaea* is proposed for this microsporidian.

The parasite and its effect on the host tissues were studied in living and fixed material. The methods by which stock cultures of *Cynaesus angustus* were established and disease-free first instar larvae obtained for experimental purposes are described. Quaker brand degerminated, yellow corn meal was used as a food medium in the experimental studies. A spore-meal mixture, for infection studies, was prepared with corn meal and finely crushed bodies of adults and/or larvae which had died of the disease.

The germination of spores and the emergence of the amoebula from the spores were observed in a salt solution, tap water, distilled water, egg albumen, and in host haemolymph. Observations on the viability of spores of various ages in the above media and their effectiveness in the transmission of the disease to new hosts were also made.

Nosema cynaea completed its life cycle within 8 days in larvae infected during the first instar; within 11 days in larvae infected in the last three instars of development; and within 10 days in adults.

The spores of *N. cynaea* were mechanically disseminated through the food medium when larvae and adults fed on the various stages of the insects which died of the disease. A mite, *Blattisocius triodons* Keegan, was also responsible for the mechanical distribution of the spores. It is believed that the feces of the host played a part in the dissemination of the spores.

New hosts became infected when the spores of *Nosema cynaea* were ingested. This is believed to be the only significant means for the spread of the parasite from host to host. Germinative infection, if it occurs, is believed to be insignificant.

The macroscopic symptoms of the disease consist of milky-white

¹ Doctoral thesis number 1026 submitted January 27, 1950.

opacity, black spots and distortion of the integument. The reliability of these symptoms in diagnosis was determined. In addition, the length of time required after larvae were exposed to infection for the black spots to appear, and how soon the larvae died after the spots appeared were determined.

The fat body cells and haemocytes of the larval, pupal and adult stages of *Cynaëus angustus* were the principal tissues attacked by the parasite. The parasite was confined to the cytoplasm of these cells. Spores were also found in the germaria of adult females. The nuclei of host fat cells and of the haemocytes became distorted, eccentric, and assumed a shapeless mass.

Studies of the effects of parasitism on larval weight were conducted. The weight of diseased larvae was found to be significantly less than the weight of healthy larvae of the same age. It was also observed that in some cases infected larvae lived longer than the time required for healthy larvae to transform to the adult stage.

Larvae, infected in the first instar, died before pupation whether they were exposed to infection for 24 hours or throughout life. However, when larvae in the last three stages of development were constantly exposed to infection they were able to transform to the adult stage in many cases.

The parasite was effective in reducing or wiping out host populations under laboratory conditions.

A SELECTION INDEX FOR BUTTERFAT PRODUCTION IN JERSEY CATTLE UTILIZING THE FAT YIELDS OF THE COW AND HER RELATIVES¹

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Several biological conditions may limit the improvement which selection can make in the hereditary worth of dairy cattle for fat production. Low degree of heritability for the trait, low selection intensity possible, and inaccuracies in selection restrict actual progress. When a breeder also selects for traits other than fat production, genetic and environmental correlations between the several traits may further limit the progress that can be realized for each.

The present study was an effort to devise an index for selecting dairy cattle more accurately by using information on the production of the individual and its relatives. The relatives considered were the cow's dam, daughters, maternal half sisters, and paternal half sisters. Information about the production of the cow and her relatives is combined in such a way that the index of an animal will be more highly correlated with its breeding value than if the information were combined in any other linear manner.

Construction of the index required reliable estimates of four fundamental statistics: the repeatability or intraclass correlation between records of the same cow, the correlation between paternal half sisters, the correlation between maternal half sisters, and heritability. These statistics were estimated from 23,330 lactation records from 12,405 cows which were in 293 Jersey herds on Herd Improvement Registry test during 1943 to 1947. The 293 herds were scattered among forty-two states. The average fat production on a 305-day, twice-a-day milking, mature equivalent basis was 429 pounds.

The analysis of variance, including the separation of the total variance into its components, was the principal analytical method. Correlations between the items included in the index were derived using Wright's method of path coefficients. The weights to be given the fat yields of the individual and its relatives were determined by using multiple correlation.

Repeatability was .412 with 10 per cent fiducial limits of .399 and .422. When the variance due to year-to-year environmental variation within herd was accounted for, repeatability was .459. Presumably this value of repeatability could not be realized in practice unless the varia-

¹ Doctoral thesis number 1021, submitted December 14, 1949.

tion attributable to environmentally-caused fluctuations of the herd average from year-to-year could be removed from the records which are actually used.

When intraherd yearly variation was accounted for, the correlation between paternal half sisters was .123. When the year component of variance was ignored, this correlation was .120. There seemed to be a strong environmental contribution to this correlation although its physical basis is not clear. The maternal half sister correlation was .090 when the influence of the year component was considered and correction was made for some of the daughters of the different dams being paternal half sisters. Ignoring the influence of the year component made the correlation .086.

Heritability was computed as twice the intraherd regression of daughter on dam. There were 4,764 daughters from 3,363 different dams in 290 herds. Heritability of differences in fat production on a single record basis was thus estimated as .201, with 5 per cent fiducial limits of .150 and .253.

Selection indexes were constructed for cows with production records and also for young animals whose own phenotype was not yet available. Two separate indexes were developed since genetic progress from selecting young stock on the best index developed especially for them would be approximately 1.20 times that to be expected from using for them the same index derived for cows with own records and deleting the items for own production and for daughters' production. If heritability is near .201, which was the value found in these data, information on the individual should receive about 2.75 times as much attention as the same information on the dam. The comparative weight to be given the other relatives varies with the number of individuals in the group and, in some cases, with whether other relatives are present.

Progress to be expected by using the index for selections between cows with records of their own would be about 1.10 to 1.15 times faster than by making selections on own performance alone. However, that ratio would depend on the number of records on the individual, on the number and kinds of relatives, and on the amount of information on each relative.

EARLY TESTING AS A MEANS OF EVALUATING F₁ HETEROSIS BETWEEN INBRED LINES OF *DROSOPHILA MELANOGASTER*¹

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Inbreeding followed by crossing has been effective in increasing yield of various species of plants and animals. The formation and selection of inbreds are the important steps to get desirable hybrids. Good combining abilities of lines are usually estimated by test crosses or top crosses. Tentative evaluation of the predictive value of such tests has been made for corn. The present work was undertaken to estimate the worth of early testing as a means of selecting inbred lines having desirable combining ability in a species of animals, *Drosophila melanogaster*.

Progenies from three strains of *Drosophila melanogaster* were used as foundation stock to establish the inbred lines utilized in these experiments. The stocks were of different geographical or chronological origins: Amherst, Massachusetts, Ames, Iowa 1943 and Ames, Iowa 1947. Matings were throughout full brother x sister in single pairs. A synthetic stock derived from eight inbred lines was used as the tester parent in the crosses. A 3-day laying period, fifth, sixth, and seventh day after emergence, was standard throughout these experiments.

Full egg-laying by the test females was encouraged by furnishing only freshly made egg-laying medium. Bottles containing laying females were kept closely grouped. The room temperature was controlled at 26°C. High summer temperatures occasionally went above this point for brief periods.

Three sets of data were collected: (1) the hybrid egg laying performance from different generation inbreds test-crossed to the synthetic stock; (2) egg yields from pure line flies after more than twenty generations of inbreeding; and (3) egg yields of single cross progeny resulting from mating individual inbred lines in all possible combinations.

Real differences in the combining ability between strains were found in first generation inbreds crossed to synthetic testers. Ames 1947 had the highest average egg record, 178.8 ± 2.5 per fly. Ames 1943 was second with 176.2 ± 2.7 . Amherst was last with 166.2 ± 2.8 as the average for its flies. The average coefficient of variation is about 35 per cent. The distributions for the egg productions of the three strains' hybrids are continuous and symmetrical. These facts support the infer-

¹ Doctoral thesis number 983, submitted July 15, 1949.

ence that a large number of genes with small additive effects are responsible for the variation.

The different strains show a similar trend. The average within-line standard deviation does not decrease much as inbreeding advances. Inbreeding did not stabilize the egg productions of the different synthetic \times inbred crosses. Instead, the egg productions of these test hybrids decrease steadily at a rate of about 2.4 eggs per generation.

The degree of heterosis exhibited by inbred lines crosses increases with the generation of inbreeding from fifteenth, twenty-fourth, and thirty-fourth generations. The average percentage increases of the hybrids over the parents are 17.2 per cent, 30.2 per cent, and 62.4 per cent in the fifteenth, twenty-fourth, and thirty-fourth generations respectively. The inbreds showed lowered vigor and productiveness as inbreeding advanced but this loss of vigor is not detrimental to the egg productions of the hybrids made from these inbreds. This agrees with the assumption that inbreeding leads to homozygosity of concealed recessive genes with deleterious effects through rare crossing over. Crossing to other lines contributes other alleles to cover up the detrimental recessives by dominants or contributes beneficial effects due to heterozygosity at that locus.

Inbred lines with more than twenty generations of brother \times sister matings show downward general trend in productivity. The average strain egg production is reduced 4.3 eggs or 3-4 per cent per generation of inbreeding. This loss is in addition to lines lost due to their inability to reproduce themselves. It would appear as if a fairly consistent number of deleterious genes were being brought to the homozygous state and reducing production.

Within synthetic \times inbred crosses, the uncontrolled variations contribute most to the variations in egg production. They show a decreasing trend with increasing inbreeding. The reduction in variation is a measure of the success of the inbreeding program and its accompanying natural selection reducing the genotypic variation within lines and sorting out genotypic environmental interactions which tend to reduce the variation in egg production. Differences between reciprocals appear due to the synthetic females being poor mothers, possibly because the progeny per cross is greater than when inbreds are used. Line differences are small and irregular. Line genotypes do not seem to have separated greatly from each other during the course of the inbreeding. Strain differences are large in the first generation. Continued inbreeding has caused an increasing separation of the strains' egg productions. The results point to some reorganization of each strain toward homozygous but different types during the inbreeding course.

Within inbred lines, after twenty generations of brother \times sister matings, the uncontrolled variances are similar in the successive generation tests. The pure inbreds have 68 per cent more line within strain variance than was observed for the inbreds \times synthetic over the period covered by the same generations. The strain differences are not signifi-

cant as contrasted with the mean squares observed for lines within strains.

No trend was found in the correlation coefficients between successive tests on the same top crosses. The top-cross tests, thus, contributed no information of value for predicting subsequent performance in later generations. On the other hand, inbreds, performances in one generation after twenty generations of the inbreeding were a better index of future performance than like tests made on top cross.

General combining ability, maternal influences, specific combining ability, and the effects of reciprocal crosses freed of additive, specific, and maternal effects show small effects on line cross performance. The uncontrolled portion of the total variance, contributes most to the variance, 60 per cent. Taking σ_e^2 as a standard for comparison, general combining ability is 23 per cent, maternal effect is 11 per cent, specific combining ability is 9 per cent, and a male effect is 46 per cent.

Correlation coefficients between general combining ability, g values, of three different generations are negative and small. Correlations for the maternal effects are small and inconsistent. Values for specific combining ability are positive and small. Those for male effect are negative and small. The lack of stable estimates for the performance of the different inbred lines suggest strong environmental-genotypic interactions of direct significance to successive yields of the same line crosses.

THE COMBINING ABILITY OF SELECTED ALFALFA CLONES AS RELATED TO THE SELF-FERTILITY OF THE CLONES, THEIR F_1 AND F_2 PROGENIES¹

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Fourteen selected alfalfa clones were individually selfed and crossed within selected groups in 1948. High and low self-fertility groups were made up of four clones each while groups of high and low combining ability were made up of five clones each. Four clones appeared in both fertility and combining ability groups.

The flowers were selfed by permitting the staminal column to strike the flat side of a toothpick upon tripping. To prevent accidental crossing, each plant had its own toothpick.

In making crosses the flowers were not emasculated, but pollen was applied to the stigma within one-half hour after the standard petal was removed and the flower tripped.

All selfing and crossing operations were carried out in the greenhouse on plants grown in 4-inch clay pots.

The parent plants used in the greenhouse in 1948 were transplanted to a randomized block yield test in the field, and seedling plants of each cross (F_1) and self (S_1) were established in a triple lattice yield test. Forage yields were taken in 1949, the first crop year.

Rooted cuttings were used to establish plants of the original parental clones and the F_1 and S_1 progenies, for selfing in 1949. Each test was set up in a randomized block design and approximately 100 flowers were selfed on each plant.

A further selfing study was made in 1950 on F_2 plants established from selfed seed of selected F_1 progenies. Six of the original crosses were selected, including two high x high, two low x high and two low x low self-fertility crosses. Each original cross was represented by approximately 144 F_2 plants, arranged in a randomized block design. Approximately fifty flowers were selfed on each plant.

Records were kept on the number of flowers selfed or crossed per raceme, pods produced per raceme, and seeds produced per raceme, as well as the date each cross or self was made.

1. Self-fertility among the fourteen parental clones varied from .14 to 2.64 seeds per flower tripped in 1949. Similar self-fertility ratings were obtained in 1948 and, in general, these ratings confirmed those obtained on the same clones in a previous study.

¹ Doctoral thesis number 1073, submitted June 6, 1950.

2. Variability in self-fertility among individual clone members often was high, suggesting the need for replication in self-fertility studies, even though the analysis of variance did not indicate a significant difference among members within clones.

3. High self-fertility clones produced approximately three times as many seeds when selfed or crossed as did the clones in the low self-fertility group indicating a relationship between self- and cross-fertility. However, there was little difference between the high and low combining ability groups.

4. Forage yields of the individual parental clones varied significantly with an F value exceeding the 1 per cent level.

5. Forage yields of the F_1 and S_1 progenies varied significantly while the S_1 progenies yielded, on the average, only 61.97 per cent as much as the F_1 progenies. Large increases in F_1 over S_1 suggested that only a small amount of selfing occurred when crosses were made without emasculation. F_1 progenies from high combining clones yielded, on the average, significantly more than those from low combining clones, but yield differences between fertility groups were not significant.

6. The self-fertility of S_1 plants within clones and of F_2 plants within F_2 progenies varied significantly, whereas that of F_1 plants within F_1 progenies did not vary significantly.

7. The rank of the parental clones in self-fertility was maintained through the F_2 generation even though the average self-fertility was drastically reduced. Many F_2 plants were self-sterile but some produced nearly as much as or more seed upon selfing than did the parental clones.

8. The self-fertility of the parental clones was not significantly correlated with the yield of parental clones, F_1 or S_1 progenies. The yield of parental clones was significantly correlated with the yield of F_1 and S_1 progenies.

THE EFFECT OF TIME, TEMPERATURE, AND PARTICLE SIZE ON THE RELEASE OF BASES FROM SOME COMMON SOIL-FORMING MINERALS OF DIFFERENT CRYSTAL STRUCTURE ¹

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A study was undertaken to determine the effect of particle size, degree of Ca and H saturation, temperature, and time on the rate of release of bases from bentonite-mineral mixtures. Accordingly, pure mineral specimens of some of the minerals commonly found in soils, were obtained. The minerals that were selected included representatives from each of the principal structural types of silicate minerals and included olivine, augite, hornblende, albite, labradorite, microcline, anorthoclase, muscovite, phlogopite, and biotite.

The minerals were pulverized in a ball mill and then fractionated in water by syphoning off the finer fractions at a time calculated from Stokes' Law. The clay fraction was separated at the .2 micron level in a supercentrifuge. The minerals appeared to undergo some mechanical breakdown due to shaking. All the minerals except hornblende dispersed on shaking in water. For hornblende only the silt fraction could be separated from the clay fraction.

The effect of fractionation on the bases in the minerals was investigated by determining the amount of total bases in the coarse clay size of the minerals and the principal bases in the ground but untreated minerals.

The effect of particle size on the release of bases from minerals was studied by determining the amounts of the principal bases released from three different size fractions incubated with H-bentonite and pure silt size quartz. The ratio of mineral: bentonite: quartz was 2:2:3. The mineral-bentonite-quartz mixtures were placed in porous bottomed crucibles and then leached with N. ammonium acetate followed by .05 M. acetic acid in order to resaturate the colloid with H. Similar leachings were repeated at intervals for 239 days. During incubation the samples were stored in a humid atmosphere maintained at approximately 26°C. The ammonium acetate leachates were analyzed for the principal bases contained in them. The acetic acid extracts were not analyzed except for the one following the second incubation period. The acetic acid leachates following the second incubation period were analyzed for the same bases that were determined in the ammonium acetate leachates.

The effect of various degrees of Ca and H saturation was studied

¹ Doctoral thesis number 982, submitted July 15, 1949.

by incubating the coarse clay fractions of the minerals with bentonite and quartz in the same ratio as was used in the particle size experiment. The mixtures were first leached with N ammonium acetate succeeded by one of the following acetate solutions: (1) .05 M. Ca and .05 M. H (1:1 Ca:H), (2) .1 M. Ca and .05 M. H (2:1 Ca:H), (3) .05 M. Ca (Ca-saturated). The ammonium acetate extracts were analyzed for the principal bases except Ca. All minerals were included in the 1:1 Ca:H treatment but only augite, albite, microcline, biotite, and muscovite were included in the 2:1 Ca:H treatment and the Ca-saturated treatment. Other than the differences in the resaturation leachate, the samples were treated the same as in the particle size experiment, including the use of the same incubation periods between leaching operations. The coarse clay fraction of all of the minerals was incubated with quartz alone in order to appraise the effectiveness of bentonite as a weathering agent. The samples were resaturated with H after the ammonium acetate extractions.

The effect of temperature on base release was observed on the coarse clay fractions of microcline and muscovite. Samples of these minerals were stored in humid atmospheres maintained at 7, 16, and 41 degrees C. The samples were treated with ammonium acetate followed by resaturation with H by .05 M. acetic acid.

The release of bases from the coarse clay fractions of olivine, microcline, muscovite, and biotite were electrodialed at a potential of 100 volts for 5 weeks. The solutions were changed weekly and the bases contained in the cathode chamber were determined.

From the above experiments the following conclusions were indicated regarding base release from minerals:

1. The hydrolysis of ground minerals is appreciable, but the extent of hydrolysis of bases varies between the different minerals and the different ions in the same mineral.
2. The release of bases from minerals increases with decreasing particle size, but the extent to which particle size influences base release varies with different minerals.
3. Increasing the ratio of Ca:H on the colloidal complex decreases the rate of base release.
4. Base release increases with temperature but the effect of increased temperature on base release decreases with time.
5. H-bentonite is an effective weathering agent but its effectiveness varies between minerals.
6. The mechanism by which bases are released from minerals by electro dialysis seems to be governed by the same limitations as released by H- and Ca-bentonite but the rates differ.
7. Fresh minerals release bases at a fast rate but this rate rapidly decreases with time. It appears that the residual primary weathering products retard the release of bases from minerals presumably by accumulating close to the weathering surface,

8. Apparently the release of bases from olivine, augite, hornblende, albite, labradorite, microcline, anorthoclase, and phlogopite is accompanied by the breakdown of the crystal lattice in those minerals. Muscovite and biotite do not become unstable when K is released, an opinion of numerous investigators.
9. The ratio of the release of two ions present in the same mineral tends to approximate the ratio in which these ions are present in the unweathered mineral, with increasing time.
10. The silt and coarse clay fractions of the K-bearing minerals studied release bases at appreciable rates. The release of K from unweathered muscovite proceeded at a much faster rate than from phlogopite, biotite, and the feldspars. All of these minerals could be important sources of K if present in soils.
11. The order with which the minerals studied release bases approximates Goldich's stability series with the exception of muscovite.

From the above observations it appears that the rates of release of bases from soils would be affected by the types of minerals present, the size distribution of these minerals, the degree of Ca saturation of the colloid, and to a lesser extent, by the temperature. Due to the lack of mineralogical data for Iowa soils no pertinent observations can be made.

COST FUNCTIONS FOR SAMPLE SURVEYS¹

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Most statisticians are aware that in designing an investigation based on a sample, the cost of the investigation and the loss resulting from errors in the estimates should be taken into account. However, no general theory of how costs vary from one sample design to another has been presented. The present study is an attempt to present theory that would enable one to make fairly accurate estimates of the field costs of various possible designs that the statistician thinks are feasible considering the nature of the data to be collected.

For surveys that cover a certain geographical area it was realized at an early date that field cost depended a good deal on the distance among n random points. That is, in most instances, one can regard a sampling unit as a point since it is, in general, small in area as compared with the total area being surveyed.

The present study differs from previous research in that it initiates the idea that for many sample surveys one cannot travel in a straight line (or airline) fashion between points but rather one has to follow the roads. In this thesis it is assumed that the network of roads forms a grid pattern and the roads are parallel or perpendicular to the boundaries of the rectangular areas. Hence it is assumed possible to travel in a rectangular or grid fashion between any two random points. The expected grid distance is easier to calculate than airline distance.

The expected minimum grid distance among three random points in a square of side a was found to be $1.121a$. However for more than three points the derivation of the expected minimum grid distance becomes very complex and seems hardly worthwhile in view of certain practical limitations on minimizing distance among random points. Unless otherwise stated, assume that all random points have a uniform space distribution over the region.

Provided the area of a region being sampled remains constant, moderate changes in the shape of the region do not greatly affect the expected distance (airline or grid) between two random points. The mean square distance² and mean grid distance may be found between two random points in any connected region where the points may have any space distribution over the region. The results of empirical investigations on minimum average distance are presented in the thesis. Mathematically an upper bound for expected minimum grid distance is found

¹ Doctoral thesis number 1056, submitted June 1, 1950.

² Mean square distance is defined as the expected value of the square of the distance between two points.

to be $1.48 \sqrt{A/n}$ where A is the area of a convex region and n the number of random points.

The ratio of expected grid distance to expected airline distance seems to be fairly constant for various values of n in regions similar in size and shape. This ratio changes slightly with changes in shape of the region being sampled. For rectangles of side ratio 1:1, 2:1 and 4:1 the expected value of the ratio of grid distance to airline distance is 1.27, 1.25, and 1.21, respectively, if one takes the points in the order of their occurrence from left to right (see next paragraph).

The expected grid distance among n points, if one does not reverse a direction in moving across the length of a rectangle ab , is

$$(n-1) \left(\frac{a}{n+1} + \frac{b}{3} \right).$$

In many surveys smaller areas within a region may be regarded as strata. If the rate of callbacks is low, the interviewer would tend to move from one stratum into the next, visiting the sampling units within each as he goes. In this case it does not seem satisfactory to minimize the distance travelled between strata. If there are m square strata in the region of area A , then the expected grid distance is

$$\frac{n+3m}{3\sqrt{m}} \sqrt{A}$$

if the interviewer does not reverse a direction. Under the assumptions of the previous sentence, the expected total distance is a minimum when one has three points per stratum. One would expect that the total distance for a stratified sample would be greater than for a random sample of the same size and over the same region. This can be proved for a certain shape of rectangle assuming that the path between the points is never reversed. If, in a rectangle, the interviewer moves without reversing a certain direction, the expected grid distance among n points for a random sample is greater than an upper limit for minimum grid distance if n is greater than 16. However for all n , provided each stratum of a certain region contains less than 14 points, the expected directional grid distance is less than an upper limit for the minimum grid distance over the same unstratified region.

If in moving over a stratum in a certain general direction the interviewer has to turn a corner and proceed in another general direction at right angles to the first, the expected increase in distance for each corner turned is given by

$$\frac{k-1}{k+1} \frac{\sqrt{k}}{2} \sqrt{\frac{A}{n}}.$$

Each stratum may have a different density function or a different rate of sampling or both. Provided the space distribution is uniform within

each stratum, the theory developed can be applied to finding the total distance among the points.

The expected airline distance of a random point from the center of the arc which forms a circle, semicircle or quadrant is $2/3$ the radius. The expected grid distance of a random point from the center of the arc which forms a circle, semicircle or quadrant is $8/3\pi$ the radius. The expected distance of a random point within a rectangle, from any fixed point, either inside or outside the rectangle, may be found. These expected distances are useful if one can assume interviewers pick the points at random (from the points in the region in which they are interviewing) either to start interviewing after leaving their homes or to break off interviewing in order to go to their homes. If one also assumes that the frequency of travel to and from home is not influenced by nearness of home and if one can estimate the number of times the interviewers will go home, then it is possible to estimate the number of miles travelled for the Home component of mileage.

The mean square distance between any pair of random points adjacent in either clockwise or counter-clockwise direction is for a circle

$$a^2 \left[1 - \frac{8}{9} \sum_{j=1}^{\text{even } (n-2)/2 \text{ or } (n-1)/2} (-1)^{j-1} \frac{n!}{(2\pi)^{2j} (n-2j)!} \right]$$

for a semicircle

$$a^2 \left[1 - \frac{8}{9} \left(\sum_{j=1}^{\text{even } (n-2)/2 \text{ or } (n-1)/2} (-1)^{j-1} \frac{n!}{\pi^{2j} (n-2j)!} + 2n! (-1)^{(n-2)/2} / \pi^n \right) \right]$$

where the last term in () parentheses is added only when n is even and for a quadrant is

$$a^2 \left[1 - \frac{8}{9} \left(\sum_{j=1}^{\text{even } n/2 \text{ or } (n-1)/2} (-1)^{j-1} \frac{n!}{\left(\frac{\pi}{2}\right)^{2j} (n-2j)!} + n! (-1)^{(n-2)/2} \left(\frac{\pi}{2}\right)^n \right) \right]$$

where the second term of () exists only if n is odd. There is reason to believe that the square roots of any of the above mean square distances are from 6.7 per cent to 9.5 per cent greater than the corresponding mean airline distances.

To estimate the between sampling unit component of travel on first call one might use either of two formulae. If there are few callbacks, one can use the formula appropriate to moving in a general direction across

strata or counties. (In this paragraph counties are merely square blocks of equal size superimposed on a rectangle which contains n randomly chosen points.) If there are many callbacks, one can follow the methods used in deriving an upper bound for grid distance. In the latter case the interviewer may be regarded as choosing at random, the order in which he visits the points in a county. Other components of mileage are Lunch, Night's Headquarters, Between S.U.'s on Second and Third Calls or More. Each of these travel components depend on perhaps two or more variables of the design of the survey. Data have been collected on these components of mileage from three Iowa farm surveys. Constants have been fitted to this data. Hence a prediction equation for these components which should be fairly accurate, within moderate changes of the design, has been found. One can also compare the travel of the Between S.U.'s on First Call component and the Home component for these three surveys with the appropriate theoretical formulae.

From the data collected in these surveys it seemed that miles per hour for the various components was fairly stable. Hence if one estimates the number of miles for these various components the number of hours for these components can also be estimated. Thus it is possible to estimate the field costs of surveys within a fairly wide range of designs. Of course many other costs depend on the design and they should be investigated. Fortunately these other costs are not so difficult to estimate.

In many cases the statistician should be balancing the cost of any particular design against the losses resulting from errors in estimate using that design. In other words, the statistician should be minimizing both cost and loss. (If the statistician does know the values of the parameters that are present in the expected loss function he can take the value of n which makes the function a minimum.) If he does not know the parameters of the distribution he is sampling, it should be possible for the statistician to make use of Wald's minimax principle in order to decide on size of sample. In some cases it has been shown that the game with nature is especially strictly determined. However in other cases the game would be strictly determined only if a randomized decision function for both nature and the statistician is postulated. The setting up of randomized decision functions has not been accomplished in the thesis.

HELMINTHOSPORIUM VICTORIAE AND OTHER GRAMINICOLOUS SPECIES¹

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Helminthosporium blight, a disease of oats affecting only Victoria derivatives, made its first appearance in 1944, and developed to epiphytotic proportions within the two years following.² The causal fungus was recognized as a new species in 1946 (1) and named *Helminthosporium victoriae* M. and M. because of its specific pathogenicity to varieties of oats possessing Victoria resistance to crown rust.

A rapid build-up of *Helminthosporium* inoculum was facilitated by the planting of wide acreages to the susceptible Victoria derivatives in all oat-growing sections of the United States. The varieties Boone, Tama, and Control, derived from crosses with the South American variety Victoria, were developed in Iowa and released in 1942, 15 years after the introduction of Victoria into this country. The Victoria-Richland derivatives showed great promise because of their resistance to rust and smut, especially to the threatening race 45 and other races of crown rust to which there had previously been no source of resistance. In 1945, 98 per cent of the oat acreage of Iowa and more than 50 per cent of the United States acreage was devoted to these varieties.

Although *Helminthosporium victoriae* had been isolated with increasing frequency from timothy seed samples in the Iowa State Seed Laboratory since 1942, and an isolate was obtained from Tama oats seed harvested in 1944, the blight was not recognized in the field until April, 1945. At this time infected seedlings in the first and second leaf stages showed reddish-tan discoloration of the blades and severe basal necrosis. Preliminary inoculations of tester varieties in the greenhouse had indicated the apparent specificity of the fungus, limited in its attack to those varieties previously mentioned. The progress of the disease was observed throughout the 1945 season in Iowa and neighboring oat-growing regions. Yields appeared to be reduced as much as 50 per cent in some fields in that year, and losses were even greater in 1946 and 1947.

The destructiveness of *Helminthosporium* blight necessitated a shift in varieties in 1947 to Bond hybrids that were available and to older varieties resistant to this disease. At the present time Victoria-Richland

¹ Doctoral thesis number 1074, submitted June 7, 1950.

² Observations and studies of this oat disease and the fungus causing it have been made over a period of 5 years at the Iowa Agricultural Experiment Station in cooperation with Dr. H. C. Murphy of the United States Department of Agriculture, Bureau of Plant Industry.

derivatives are grown only in a few areas where climatic conditions limit the severity of blight, and in regions where the new Bond derivatives are not agronomically suitable. The rapid development of this disease has illustrated the dangerous potentialities of planting wide adjoining acreages to varieties of similar disease-resistant types, utilizing only one source of resistance to a certain disease or group of diseases.

Seedlings grown from seed infected with *Helminthosporium victoriae* showed severe blighting at the time of emergence, and usually died in the first or second leaf stage. A large percentage of pre-emergence killing occurred when badly infected seed was planted. Seed treatment as a means of control was found to be effective only to the extent of retarding infection, older plants succumbing more slowly than those infected at the time of seed germination.

Characteristic symptoms of seedling infection in the field were pinkish-brown necrosis of the white mesocotyl region, and longitudinal brownish striping of the blades or over-all reddening. The striping was evident in the lower leaf first, progressing upward to subsequently developed leaves until the plant was killed. The fungus was not isolated from the striped blades until after death and drying of the tissue, or after the leaves had come into contact with moist soil. The leaf striping and basal necrosis were also typical of infected plants in later stages of maturity. The basal stem- and root-rot were the most dependable diagnostic symptoms, however, since leaf discoloration may often be due to several causes. Nodes of mature plants in the field were blackened with abundant sporulation of the fungus, and the lower internodes showed a characteristic brownish translucence. Culms weakened by severe infection commonly broke over near the ground line and at the lower nodes, resulting in excessive lodging.

Symptoms expressed in plants of susceptible varieties grown in artificially infested soil in the greenhouse were comparable to those observed in the field, and varietal reaction readings made in the greenhouse were in complete agreement with field data. Greenhouse inoculations (using several techniques) of some 300 oat varieties and selections showed no exceptions to the rule that oat varieties possessing Victoria-type resistance to race 45 (and certain other races) of crown rust are susceptible to *Helminthosporium victoriae*, and varieties lacking this crown rust resistance are resistant to *H. victoriae*. A preliminary inheritance study (3) showed susceptibility to *H. victoriae* to be dominant in a simple three-to-one ratio.

Production of a thermostable toxin by this fungus was demonstrated (2) in experiments using heat-killed mycelium as inoculum. This toxic substance is apparently responsible for the characteristic foliar striping and reddish-tan discoloration some distance in advance of the growth of the fungus.

In the course of the Iowa investigations, *H. victoriae* was compared morphologically and pathologically with numerous other species. Comparisons were made especially with those species most familiar to plant

pathologists (including those found on oats), and with species most closely resembling *H. victoriae* in spore morphology according to their descriptions in the literature. A discussion of the significant taxonomic characters for distinguishing species has been presented in the original paper, and a detailed comparison of species made. *H. setariae* Saw. is considered to be the species most similar to *H. victoriae*, but *H. sativum* is the species most often confused with the new species, probably because of the similarity of atypical spores on weathered plants in the field.

The definite greenish color of young but fully developed spores of *H. victoriae* is the most evident character that distinguished this species from similar forms. Spores become darker green to olivaceous with age. The maximum diameter occurs at or near the middle, the contour tapering toward each end, more strongly toward the apex. The basal end is rounded more broadly than the distal end. This difference in diameters, however slight in many spores, is one of the most typical characters of this species. Normal conidia measure 40–130 (70) μ x 11–25 (15) μ with 4–11 (8) septa, have moderately thin walls, and germinate by one polar germ tube from each terminal cell. Typical cultures form a light to medium-gray tufted colony on oat agar. Three profusely sporulating saltant strains have arisen in culture, two of these showing tendency toward perithecial production. Conidiophores are comparatively short.

H. victoriae has been isolated from a number of grass hosts, mainly from seed. The fungus was found fruiting on germination blotters of these seed samples, especially those of timothy, sorghum cane, and bahia grass. The fungus was not pathogenic to these grasses beyond the petri dish germination stage. *H. victoriae* was nonpathogenic to all varieties of oats other than *Victoria* derivatives, and to barley, wheat, and *Setaria* spp.

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ANALYSIS OF BERYLLIUM-ALUMINUM MIXTURES AND PROPERTIES OF COMPLEXES INVOLVED ¹

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The use of ultra-violet spectrophotometry for quantitative inorganic analytical purposes has long remained unexploited. An extremely large number of complexes of metals has characteristic absorption properties in the visible portion of the spectrum and many colorimetric methods of analysis depend upon this fact. With the present universal availability and use of ultra-violet spectrophotometric equipment the characteristic absorption properties of many "colorless" metallic complexes in the ultra-violet region of the spectrum should just as conveniently be capable of similar utilization.

The rather complex aqueous chemistry of beryllium has long been in need of elucidation. Also, due to the ever increasing industrial importance of beryllium, its analytical chemistry is in need of extension and closer examination. It was with these facts in mind that the present work was carried out.

In this work a new spectrophotometric method for the determination of beryllium was described. The method depends upon the increased absorption at 317.0 mμ which is brought about by the addition of beryllium to solutions of sulfosalicylic acid in the pH range of 9.2 to 10.8. Comparisons were made between known 5-sulfosalicylic acid and sulfosalicylic acid from two different commercial sources to show that the commercial materials were either, in reality, the 5-sulfosalicylic acid or, at least as far as complex formation with beryllium is concerned, they behaved the same as the 5-sulfosalicylic acid. These comparisons were made with respect to the potentiometric titrations with alkali of equal molar solutions of the acids, the ultra-violet absorption spectra of equal molar solutions of the acids at the same pH, and the ultra-violet absorption spectra of equal molar solutions of the acids containing equal quantities of beryllium at the same pH.

By means of the method of continuous variations, developed by Job, it was shown that the ratio of sulfosalicylate to beryllium in the complex is 2 to 1. The constancy of the maximum composition at various wave lengths indicated that only the one complex is present in appreciable quantities at the optimum pH.

An alkalimetric titration of pure beryllium basic acetate, $\text{Be}_4\text{O}(\text{C}_2\text{H}_3\text{O}_2)_6$, in solutions containing a large excess of sulfosalicylate, was employed to show that at the optimum pH of complex formation,

¹ Doctoral thesis number 1044, submitted March 28, 1950.

both of the hydroxylic hydrogen atoms of the sulfosalicylate ions are removed in the formation of the complex. The beryllium-sulfosalicylate complex ion was therefore believed to be best formulated as $\text{Be}(\text{C}_6\text{H}_3\text{SO}_3\text{OCO}_2)^{\equiv-}_2$. Qualitative theoretical justification for this formulation was presented.

The molecular extinction coefficient at 317.0 m μ . for the free sulfosalicylate ion, $\text{C}_6\text{H}_3\text{SO}_3\text{OHCO}^{\equiv-}_2$, was found to be ca. 691 through the pH range of interest; by a method of successive approximations that of the beryllium-sulfosalicylate complex, $\text{Be}(\text{C}_6\text{H}_3\text{SO}_3\text{OCO}_2)^{\equiv-}_2$, was found to be ca. 7750. Knowing the molecular extinction coefficients of these two absorbing species it was found possible to evaluate the equilibrium constant at the optimum pH for the dissociation of the complex ion to form free sulfosalicylate ions, $\text{C}_6\text{H}_3\text{SO}_3\text{OHCO}^{\equiv-}_2$, and an unknown beryllium species, Be_x . The value obtained was approximately 2.1×10^{-9} at $25 \pm 1^\circ\text{C}$.

The spectrophotometric determination of very small amounts of beryllium in aluminum by means of the sulfosalicylate complex was successfully carried out. Aluminum was complexed with ethylenediaminetetraacetic acid to eliminate its interference. A method of analysis for beryllium in aluminum was proposed. In this method a solution containing 0.2000 ± 0.0200 g. of aluminum is used and concentrations of beryllium in aluminum up to 0.23 per cent are determinable. Under the most favorable conditions concentrations as low as 0.0015 per cent beryllium in aluminum should be detectable. The relative errors for such low beryllium concentrations are quite great. It was indicated that by varying the sample size, extent of dilution, etc., widely differing ranges of concentrations of beryllium in aluminum might be accommodated.

In general a net change of ca. 0.01 optical density units is brought about by varying the pH from 9.2 to 10.8 for a particular beryllium concentration. For very low beryllium concentrations it is best to adjust the pH of sample solutions, reference solutions, and standard solutions to as nearly the same pH as possible. This is conveniently done by using concentrated reagent-grade ammonium hydroxide for the adjustment of pH. The ethylenediaminetetraacetic acid very effectively buffers the solutions in the vicinity of a pH of 10.0.

The degree of interference brought about in the proposed method by the presence of 1.0 per cent, 0.0020 g., of each of a number of cations was determined. This was done for beryllium concentrations in aluminum of 0.0090 per cent and 0.135 per cent. In the lower beryllium concentration appreciable interferences were found for cupric copper, ceric cerium, and ferric iron. Much smaller interferences were found for stannous-stannic tin, zirconium-hafnium, and nickel. Interferences by chromic chromium, manganous manganese, and cobalt were small enough to be neglected. No interferences were observed for sodium, potassium, magnesium, calcium, zinc, strontium, cadmium, barium, lanthanum, titanous titanium, plumbous lead, arsenous arsenic, and antimonous antimony. In the higher beryllium concentration appreciable

interferences were found for cupric copper, ceric cerium, hafnium-zirconium, and ferric iron. No interferences, or at least negligible interferences, were observed for sodium, potassium, magnesium, calcium, zinc, strontium, cadmium, barium, lanthanum, titanous titanium, stannous-stannic tin, plumbous lead, arsenous arsenic, antimonous antimony, chromic chromium, manganous manganese, cobalt and nickel.

The interference in the proposed method due to iron was successfully eliminated by extraction of the ferric chloride with freshly distilled β,β' -dichloroethyl ether.

It was shown that relatively large amounts of perchlorate, sulfate, chloride, and fluoride ions do not interfere in the proposed method. Large amounts of acetate ion interfere slightly, and phosphate and nitrate ions must not be present in appreciable quantities.

PHYSIOLOGIC RESPONSES OF *BROMUS INERMIS*

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Fertilizer and clipping studies were conducted on sod-bound brome-grass plots at the S.C.S. Nursery in 1947 and 1948, and on a new seeding of brome-grass at the Agronomy Farm in 1948. Treatments consisted of all possible combinations of the following: (1) Fertilizer: 400 pounds ammonium sulfate applied early fall; 400 pounds ammonium sulfate in early spring; 200 pounds ammonium sulfate in early fall and 200 pounds ammonium sulfate in early spring; unfertilized. (2) Clippings: clipped fall only; clipped spring only; clipped fall and spring; unclipped.

For both years, forage yields were increased approximately two times by the fall application and three times by the spring application of nitrogen fertilizer. Average yields of forage for the four fertilizer treatments were reduced little by fall clipping but approximately 50 per cent by spring clipping. The addition of seasonal clippings to the seed harvest forage yields did not change the relative yield of forage under the various fertilizer and clipping treatments. Differences in yield due to fertilizer, clipping treatments, and to their interaction were significant at the 1 per cent level. There was no significant difference between years. On the new seeding of brome-grass at the Agronomy Farm in 1948, forage yields were increased by nitrogen fertilizer, and spring clipping reduced forage yields 50 per cent. The average fertilizer effect on the fall, spring, and fall and spring applications was approximately the same on new seeding. Differences in yields due to fertilizer and clipping treatments and to their interaction were significant at the 1 per cent level.

Nitrogen applied in the spring increased seed yields eight times over the unfertilized plots in 1947 and three times in 1948 while the fall, and fall and spring applications were less effective. Fall clipping did not reduce seed yields but spring clipping reduced yields 50 per cent in 1947 and 30 per cent in 1948. Differences due to fertilizers, fertilizers x years, and clippings were significant at the 1 per cent level, and differences due to years and clipping x fertilizers were significant at the 5 per cent level. On new seeding, in contrast to old stands, fall application of nitrogen was more effective in increasing seed yields than spring. Spring clipping was again the more detrimental, reducing seed yields approximately 50 per cent. Differences due to fertilizers,

¹ Doctoral thesis number 1038, submitted March 13, 1950.

clippings, and clippings x fertilizers were significant at the 1 per cent level.

Fertilizer studies were also conducted at the Soil Conservation Farm, Clarinda, Iowa. In the first experiment, 1948, treatments were as follows: (1) unfertilized; (2) 300 pounds 20 per cent superphosphate; (3) 60 pounds ammonium nitrate; (4) 60 pounds ammonium nitrate plus 300 pounds 20 per cent superphosphate; (5) 120 pounds ammonium nitrate; (6) 120 pounds ammonium nitrate plus 300 pounds 20 per cent superphosphate; (7) 180 pounds ammonium nitrate; and 180 pounds ammonium nitrate plus 300 pounds 20 per cent superphosphate. At Clarinda seed yields were increased, as in the previous experiments, by nitrogen fertilizer, but the addition of 20 per cent superphosphate increased seed yields only at the higher rates of nitrogen application.

In the second experiment, 1949, treatments were as follows: (1) unfertilized; (2) 60 pounds ammonium nitrate applied early fall; (3) 60 pounds ammonium nitrate in early spring; (4) 120 pounds ammonium nitrate in early fall; (5) 120 pounds ammonium nitrate in early spring; (6) 240 pounds ammonium nitrate in early fall; (7) 240 pounds ammonium nitrate in early spring; (8) 60 pounds ammonium nitrate in early fall plus 60 pounds ammonium nitrate in early spring. Data from these experiments indicate that the spring application of 20 pounds of nitrogen was more effective than fall. There were no differences between fall, spring, or fall and spring application when ammonium nitrate was applied at the rate of 40 to 80 pounds.

In the study of plant characters at the S.C.S. plots in 1947 and 1948, and at the Agronomy Farm in 1948, nitrogen fertilizer increased the number of panicles per unit area, spring application being more effective than fall in the old stand of brome grass and little difference between fall and spring applications in the new seeding. Clipping treatments had little influence on the number of panicles produced. In general, nitrogen fertilizer increased panicle length while spring clipping decreased panicle length. Nitrogen fertilizer increased the total number of florets per panicle while spring clipping decreased the number. Nitrogen fertilizer decreased the percentage of fertile florets but increased the weight of threshed seed, while clipping decreased the weight of threshed seed per panicle.

Plots at the Agronomy Farm and at the Clarinda station were continued to study the residual effect of the fertilizer treatments on forage and seed yields in 1949. At Clarinda, there was a carry-over of the nitrogen fertilizer only at the higher rate, 180 pounds of ammonium nitrate (60 lbs. N). No residual effect of nitrogen fertilizer was observed in 1949 on the plots at the Agronomy Farm.

The interaction of photoperiod, temperature, clipping treatments, and nitrogen fertilization in the induction and development of flower primordia in the greenhouse in 1946 and 1947 was studied. In the two years, 1946 and 1947, of the ninety-six brome grass panicles, all were produced under an induction of normal fall photoperiods. All but fifteen

were produced under outside temperatures, all but fifteen in fertilized pots and all but three when unclipped.

In general, more culms were produced under normal fall than under 18-hour photoperiods. Clipping treatments reduced the number of tillers. In the majority of cases greenhouse temperatures and nitrogen fertilization increased the number of culms per pot.

Unclipped plants showed higher top weights under 18-hour photoperiods. On the average, both root and top weights were higher under the longer photoperiods and greenhouse temperatures. Nitrogen fertilization increased root and top yields while clipping reduced them. Among unclipped plants, the top-root ratios were greater under the 18-hour photoperiods, greenhouse temperatures, and nitrogen fertilization. Top-root ratios were much higher in unclipped than in clipped plants.

In both the greenhouse experiments and the fertilizer and clipping experiments in the field, food reserves of bromegrass were measured to determine the effect of various treatments on plant reserves. In general, food reserves in the greenhouse were increased by nitrogen fertilizer and cool temperatures. Photoperiod had little effect but clipping reduced food reserves. In the field food reserves were the highest on fertilized and clipped plots. Food reserves were decreased from fall to spring. Differences in field reserves due to treatment were not significant. Over-winter losses were high in sucrose, soluble polysaccharides and acid hydrolyzable materials, with the highest percentage loss shown for soluble polysaccharides.

VARIATION OF OIL PERCENTAGE IN THE CORN KERNEL AND A STUDY OF ITS INHERITANCE THROUGH THE USE OF CHROMOSOMAL TRANSLOCATIONS¹

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Corn oil is the most valuable, per unit weight, of any major product of the corn milling industry. It also has a high caloric value indicating then an increase in the oil percentage of corn grain would be of value from the standpoint of livestock feeding.

It is a well-established fact that the corn breeder can modify the percentage of oil in corn grain. Several problems, however, confront the plant breeder in his attempts to improve the milling and feeding qualities of corn grain by increasing its oil content. In both the wet-milling and dry-milling processes only the oil in the germ is commercially extractable. Oil in the endosperm is not recovered, and if present in too high percentage even may hinder the efficient separation of starch and gluten. Thus in addition to a study of factors influencing the total oil content it also is desirable to consider the distribution of that oil in the component parts of the corn kernel. For the over-all improvement of the milling and feeding qualities of corn grain it is necessary to consider the effect of increased oil percentage on both the quantity and quality of the protein in the kernel. Feeding tests have shown that the protein in the germ portion of the corn kernel is nutritionally balanced. Endosperm protein, on the other hand, is of poor quality, being deficient in two of the essential amino acids, lysine and tryptophan. Information is thus needed on the effect of increased oil percentage on the distribution of protein in the corn kernel. Since tryptophan is one of the amino acids for which corn protein is deficient, it also is of interest to consider the effect of increased oil percentage on the tryptophan content of the grain.

Corn samples of self-pollinated ears from 215 F_2 and backcross plants of a cross between a low oil strain (1.3 per cent oil) and a high oil strain (12.0 per cent oil) were hand separated into their germ and endosperm portions. Each portion was analyzed for percentage oil, percentage protein, and relative concentrations of tryptophan. Correlation and regression analyses were employed to study the interrelations among certain measurements indicating the variation and distribution of each of these constituents in the corn kernel.

Laboratory analyses for total oil content are less expensive and

¹ Doctoral thesis number 1030, submitted March 7, 1950.

more rapid than those for germ oil percentage which entail hand dissection of the kernels. Variation in the percentage of germ oil in the whole kernels, however, was very closely associated with variation in total oil percentage. This indicated that whole grain analyses for oil percentage would be satisfactory as a basis for selections in a breeding program directed towards increasing the commercially recovered germ oil of the grain. From appropriate correlation and regression coefficients, it was apparent that increased oil content resulted primarily from (a) increased proportion of germ and (b) increased concentration of oil in the germ. Thus, as percentage oil in the whole grain increased, the major portion of that increase was of the commercially recovered germ oil.

Variation in total oil content of the corn kernel was only slightly associated with variation in concentration of oil in the endosperm. The data thus indicated that it should be possible to select corn strains with increased total oil content which do not have a commercially undesirable level of oil in the endosperm.

Variation in total oil percentage was not appreciably associated with the variation in total protein percentage. Total oil percentage, however, was positively correlated with percentage germ protein ($r = +.36$ to $+.50$) and relative concentrations of tryptophan in the corn kernel ($r = +.42$). Thus it appears that increased oil percentage also may result in increased percentages of relatively high quality protein in corn grain. This positive relationship is apparently accounted for by the rather close association existing between each of these variables and proportion of germ.

Dr. E. G. Anderson of the California Institute of Technology has suggested the use of chromosomal translocations (chromosomal interchanges) for investigating the linkage relations of genes conditioning quantitatively inherited characters. The positions of the breaks involved in the interchanges are used as chromosome markers for such studies. Each interchange may be considered to test a portion of the chromosome not exceeding forty to fifty genetic units on either side of the break locus. Chromosomal interchanges in the heterozygous condition result in the abortion of approximately 50 per cent of both the pollen and ovules in maize. Such plants are said to be semisterile. Crosses between normal and semisterile maize plants give equal numbers of normal and semisterile offspring.

The chromosomal interchange stocks used in this study were 1-9c, 2-9b, 3-9a, 4-7a, 5-9, 6-9a, 8-9, and 9-10b. Each of these stocks was crossed with a high oil strain and a low oil strain. The F_1 plants, heterozygous for the chromosomal interchanges, were backcrossed to their respective low oil or high oil parent. These backcross progenies were thus segregating for semisterility as well as for genes conditioning oil percentage. A significant difference in the oil content of the kernels from normal plants as compared to that of kernels from semisterile

plants would indicate linkage between either or both chromosomal interchange points and a gene or genes conditioning oil percentage.

The results in the high oil backcross progenies suggested that genes conditioning oil percentage were present in each of the chromosome regions tested. The genetic effects of each of these chromosome regions, however, were small and of about the same magnitude as any of the other regions tested. In the low oil backcross progenies there was little evidence of any associations between genes conditioning low oil content and the points of chromosomal interchange involved. In these low oil progenies it was postulated, however, that if there were a rather large number of genes concerned, the effects of the genes in any one chromosome region would be too small to be accurately evaluated by this interchange technique.

The observed results in both the high oil and low oil backcross progenies were compared with those that might be expected under various assumptions as to the number and distribution of genes conditioning oil percentage. It appeared that the observed data were compatible with the hypothesis that the oil content of the strains tested was conditioned by a rather large number of genes, having small and approximately equal effects, and distributed at random over the ten pairs of maize chromosomes. It should be emphasized, however, that the data are only suggestive, and should not be construed as a critical test of such a hypothesis.

SELECTION FOR ECONOMIC CHARACTERS IN MERINO SHEEP ¹

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The main purpose of the study was to explore and clarify some of the problems of selection confronting the breeder of Australian Merino sheep, and if possible to suggest measures to improve the efficiency of selection.

Records of greasy fleece weight, yield, clean fleece weight, staple length, body weight at 10 months, skin fold score, and fiber fineness were studied. Data were obtained from the flock of Merino sheep at Trangie Experiment Farm, New South Wales, Australia, between 1943 and 1948.

Phenotypic means, variances, and correlations were calculated. Mean values of greasy and clean fleece weight, yield, and staple length were higher than those reported in the literature for American wool-producing breeds. The average body weight at 10 months was approximately the same as that of comparable American breeds at weaning.

	Yield	Greasy Fleece Weight	Clean Fleece Weight	Staple Length	Body Weight	Folds	Crimps	Fiber Diameter
Yield.....05	.69	.47	.04	-.25	-.30	.07
Greasy Fleece Weight.....	.0577	.22	.30	.31	-.25	.15
Clean Fleece Weight.....	.69	.7748	.25	.06	-.36	.14
Staple Length.....	.47	.22	.4812	-.34	-.34	.03
Body Weight.....	.04	.30	.25	.12	-.12	.00	.15
Folds.....	-.25	.31	.06	-.34	-.1209	.01
Crimps.....	-.30	-.25	-.36	-.34	.00	.09	-.30
Fiber Diameter....	.07	.15	.14	.03	.15	.01	-.30
Degrees of Freedom....	445	445	445	445	445	445	359	210

¹Doctoral thesis number 1067, submitted June 3, 1950.

Coefficients of variability for fleece weight, staple length, and body weight were fairly constant. Variances among groups of rams, ewes, and wethers in different years were heterogeneous, but of the twenty-eight sets of correlation coefficients, only one, that between staple length and body weight, was significantly heterogeneous. The correlation coefficients were:

Repeatability values were found as follows:

Greasy fleece weight	0.74
Yield	0.54
Clean fleece weight	0.69
Staple length	0.71
Body weight — rams	0.78
Body weight — ewes	0.71
Folds	0.69

The 95 per cent confidence intervals were of the order of ± 0.05 to ± 0.09 . The values for repeatability indicate that repeated observations on the same character have little value in a breeding program, unless they can be made cheaply and do not delay decisions.

The following relative values were assigned to characters considered of economic importance. Because suitable data were not available these values are only tentative.

One pound of clean fleece weight	10 units
One centimeter of staple length	1 unit
One pound of body weight	1 unit
One grade of skin folds	-5 units
One crimp per inch	2 units

Heritability was calculated from the regression of offspring on parent, with the following results and standard errors:

Greasy fleece weight39 \pm .10
Yield75 \pm .23
Clean fleece weight62 \pm .27
Staple length22 \pm .22
Body weight36 \pm .23
Folds47 \pm .16
Crimps per inch40 \pm .15

Genetic correlations among the characters considered in a selection index were:

	Clean Fleece Weight	Staple Length	Body Weight	Folds	Crimps
Clean Fleece Weight.	-.38	-.06	.28	-.83
Staple Length.....	-.38	-1.11	-.65	-.18
Body Weight.....	-.06	-1.1123	.12
Folds.....	.28	-.65	.2350
Crimps.....	-.83	.18	.12	.50

The genetic correlations obtained indicate that progress from simultaneous selection for these characters must necessarily be limited by unfavorable correlated responses, if the values found are approximately correct. The limited data available make any individual estimate unreliable, and some of the values obtained were shown to be impossible by multiple correlation analysis.

Unlikely values for genetic correlations were replaced by figures which appeared more reasonable, but which were chosen somewhat arbitrarily. A selection index for economic value intended to maximize genetic improvement in over-all merit was calculated. The index is

$$I = 100 + 1.54 X_1 - 1.26 X_2 + .01 X_3 - .75 X_4 + .13 X_5$$

where X_1 = clean fleece weight in pounds

X_2 = staple length in centimeters

X_3 = body weight in pounds

X_4 = skin fold score

X_5 = crimps per inch

Selection on the basis of this index should give a genetic change of .61 X_1 , — .18 X_2 , .31 X_3 , — .26 X_4 , and — .17 X_5 in the breeding values of individuals selected, for each standard deviation by which their indexes exceed the average of the unselected population.

An examination of the possibilities of the progeny-test indicated that progeny-testing is unlikely to be of much value in small flocks. Selection based on a combination of individual merit and merit of half-sibs should result in approximately the same improvement as that achieved by optimum use of progeny-tested sires, without the handicap caused by a longer generation interval. In large flocks, extensive use of outstanding sires by artificial insemination may increase progress by a factor of 1.2 compared with mass selection, for traits with heritability about 0.3, and rather less for more strongly inherited characters. The advantages gained by progeny-testing and artificial insemination are not likely to be spectacular when compared with the improvement possible from combination selection.

UTILIZATION OF EGG PROTEIN BY WELL-NOURISHED AND UNDERNOURISHED RATS¹

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The present study was concerned with a comparison of the manner in which standardized undernourished and well-nourished adult male albino rats utilized the proteins of dehydrated defatted whole egg powder when it was incorporated into the basal diet in proportions ranging from approximately 1.5 to 25.0 per cent by weight. The response was evaluated in terms of changes in body weight, retention of dietary nitrogen, the blood picture, and the size and composition of certain organs, particularly the liver.

Analysis of changes in average body weights during the balance periods of the animals in the subgroups in each series gave the first clue in respect to the way undernourished and well-nourished animals utilized the supply of egg protein in their diets. In the undernourished group, the lower percentage of dietary protein such as 1.5, 2.6, or 3.0 per cent were not quite enough to permit gains in body weight. Thereafter additional dietary protein was reflected in very good gains, the maximum increment being made when the diet contained 18 per cent of egg protein.

The picture of well-nourished rats was different when they received graded dosages of dietary protein. Gains in general were less than in animals that were partially depleted of body stores of nitrogen, indicating a lesser need for dietary protein by the well-nourished animals.

The results showed that the undernourished animals seemed to retain nitrogen in direct proportion to the quantity present in the diet until the ration contained 6.7 per cent of egg protein by weight. Then the rate of retention dropped. The retention of nitrogen was higher in undernourished than in well-nourished ones at approximately the same level of protein intake. It is interesting to point out that the depleted animals attained nitrogen equilibrium at an intake of nitrogen considerably lower than that required by the well-nourished rats, that is, 325 vs. 575 mg. of nitrogen. This indicates how eagerly the body utilizes dietary nitrogen for repletion of body stores. It seems again that the well-nourished rats expended nitrogen much more freely than the undernourished ones as judged by the urinary excretion of this element. Also, the undernourished rats excreted nitrogen in nearly constant quantities even though the protein in the diet was increased. The ability of egg protein

¹ Doctoral thesis number 986, submitted July 21, 1949.

to spare body tissues was not very evident in the well-nourished animals. When the undernourished rats were absorbing 990 mg. of nitrogen they excreted 383 mg., or nearly 40 per cent of the amount absorbed. On the other hand, the well-nourished animals absorbed 1,173 mg. of nitrogen and excreted 898 mg. or nearly 80 per cent of that absorbed. When the diet contained 18 per cent of protein, the respective percentage values were 50 and 80 for the undernourished and well-nourished rats. Results from the present investigation showed the effect of different quantities of protein intake on the moisture content of the organs. Reduction of protein in the diets of the well-nourished rats caused an increase in the amount of water present in the liver to 71.1 per cent at 1.5 per cent of egg protein in comparison with 68.8 per cent in the normal controls fed the Steenbock XVII diet. Feeding egg protein to the undernourished rats seemed conducive to the maintenance of a high moisture content of the liver. The average value for all groups was 71.5 per cent. It is probable that continued maintenance on the various egg diets would lead to a reduction in the moisture contents of the livers of rats in this series.

The data showed the effect of different quantities of protein intake on the distribution of total protein within the body. It was found that the quantity of nitrogen in the liver increased in both series as the nitrogen balance increased. The change was more marked in the undernourished than in the well-nourished ones. In the undernourished animals, the quantity of nitrogen in the liver was directly referable to the nitrogen balance until positive balances of 200 to 300 mg. were attained. Then the nitrogen concentration was maintained at a fairly constant level even though the balance reached 1,000 mg. Thus it was demonstrated that nitrogen equilibrium, often used as a criterion for adequate protein nutrition, did not accomplish the maximum storage in the liver that well may serve in increasing the body resistance in time of emergency.

Less fat was present in the livers of both series as the nitrogen in the diet was increased. The data from this experiment showed that decrease of dietary protein and thereby reduction of nitrogen retention, caused formation of livers decidedly richer in fat, even though the fat intake was not varied. Proteins seemed to be directly involved in the control of the deposition of fat in the liver.

The effect of realimenting the undernourished rats on the concentration of serum protein was quite striking. When the rations furnished proteins equivalent to 1.5 to 4.6 per cent of the diets, the relative concentrations of protein in the sera did not shift from the value of 6.7 per cent which characterized the animal receiving no protein. Thereafter, with increments in dietary protein up to 12.3 per cent, the concentrations of serum protein in the blood increased. Above this point the amount of serum proteins in the blood dropped. This situation seems to suggest a relation between the diet and the changes in concentration of liver nitrogen. In the first four groups of animals, which ingested 1.5 to 4.6

per cent of egg proteins, the concentration of serum proteins in blood remained relatively constant, and a continuous repletion of hepatic nitrogen occurred. This response seemed to have, therefore, a priority value over the generation of serum protein.

The feeding of egg diets appeared to have a stimulating effect on the production of red cells. Enrichment of diets with egg protein did not induce hemoglobin formation to any great extent. Feeding of egg diets to well-nourished rats seemed to increase the blood volume. However, this statement needs confirmation by carcass analysis for fat, moisture, and nitrogen. With the data available, it is evident that repletion of an undernourished animal resulted in a quick increase in blood volume. This may be due to hydration of the blood, or it may represent an artefact due to the differences in the composition of the animal carcass.

EXPERIENCE OF THE FEDERAL LAND BANK WITH LOANS IN FOUR NORTH CENTRAL IOWA COUNTIES, 1917-47¹

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Credit has been successfully employed by many farmers in providing the large amounts of capital essential to successful farming. Numerous farmers have purchased farms with the aid of credit and have maintained continued farm ownership. Others, however, have failed, thereby being deprived of farm ownership in addition to losing accumulated savings.

This study analyzes experience of the Federal land bank with loans closed 1917 through 1947 in Wright, Franklin, Hamilton, and Hardin counties in North Central Iowa, so that such experience can be observed and evaluated more effectively in determining appraisal and loan policies which will aid farmers in attaining continued farm ownership. A total of 2,806 loans for over 27 million¹ dollars were closed in the four counties during the 30-year period. About one-fourth were closed in the period 1917-32 and nearly one-half in the period 1933-36. Experience with these loans is well advanced.

To obtain greater uniformity in quality of farms, loans were grouped according to areas established in conformance with a national pattern of land classification, the key to which is desirability as a place to earn a living, to live, and to farm. The national pattern embodies five classifications, the most desirable being Area I and the least desirable Area V. Large parts of the four counties fall in Area I, with some in each of Areas II and III.

Repayment of loans generally has been ahead of schedule. While most loans were made for a period of 34½ years, many have been repaid as the following data indicate.

	Per Cent of Loans Repaid as of			
	March 31, 1942		January 1, 1948	
	1917-32 Loans	1933-41 Loans	1917-32 Loans	1933-47 Loans
Area I.....	55.6	22.6	83.2	74.4
Area II.....	53.5	20.5	86.5	71.9
Area III.....	41.3	13.7	64.1	64.9

¹ Doctoral thesis number 975, submitted June 27, 1949.

Repayments during the recent high farm income period were unusually large, but even before World War II many loans had been paid. Repayments in Area III have lagged behind those in Areas I and II.

Practically all foreclosures resulted from 1917-32 period loans. Only four loans made following 1932 broke down. The proportion of 1917-32 loans foreclosed and gain or loss per \$100 loaned during that period is as follows:

	Proportion Foreclosed	Gain or Loss
Area I.....	7.8 per cent	\$0.98 gain
Area II.....	6.5 per cent	0.69 gain
Area III.....	22.8 per cent	3.50 loss

The percentage of loans foreclosed was high, particularly in Area III, but a gain was realized on many acquisitions indicating factors other than value of the security were involved.

If none of the cases where a gain was realized had been acquired, the proportion of 1917-32 loans foreclosed and the resulting loss would have been as follows:

	Proportion Foreclosed with Loss	Loss per \$100 of All Loans
Area I.....	2.1 per cent	\$0.39
Area II.....	3.0 per cent	0.64
Area III.....	17.4 per cent	3.84

The proportion of loans foreclosed with loss was small in Areas I and II but large in Area III. Loss per \$100 loaned was small in Areas I and II, and moderate in Area III.

Sales of the foreclosed farms were scattered over the years 1932-43, with some concentration in the period 1935-39. Only one farm was sold after 1941, and, therefore, farm income of the war period had little, if any, influence upon gains or losses. On the contrary, acquired farms were sold during a period when land prices were lower than at any other time between the two world wars — and considerably below the immediate pre-World War I period.

All foreclosures occurred during the period 1930-41, the majority falling in the period 1931-35. Exceptionally low farm income and other economic disturbances resulting from depression and drouth were major factors contributing to breakdown of most of the foreclosed loans.

Land bank fieldmen reviewed loans at time of foreclosure and indicated factors that contributed to their breakdown. Tabulation of factors other than depression and drouth, which affected all farms, indicates heavy debts, almost invariably composed of junior and/or chattel mortgages, were a major factor contributing to foreclosure. In a number of

cases the commercial bank holding the second mortgage had closed forcing foreclosure by the land bank to protect its investment. If the land bank loan had been larger in such cases the second mortgage might not have been necessary or possible, and foreclosure might have been avoided.

Transfer of the loan due to sale of the farm or death of the borrower was given as a factor in a large proportion of the cases, particularly in Area III. Poor management and unusually poor crops were relatively important in Areas II and III. Financial irresponsibility was a factor in Area I.

Delinquency was a relatively unimportant factor contributing to foreclosure, particularly in Areas I and II. In the four counties, forty of the sixty-eight loans foreclosed were delinquent less than 10 per cent of the face amount of the loan. Only seven of the sixty-nine loans were delinquent more than 14 per cent.

Loans grouped according to amount loaned per acre show a tendency for the foreclosure rate to increase with increases in loan per acre, but the trend is not clear-cut. When the cases foreclosed with a gain are omitted it is evident the larger-per-acre loans were foreclosed with loss less frequently than the smaller-per-acre loans. In each area, per cent of 1917-32 loans foreclosed with loss was smaller in the \$100-per-acre bracket than in some lower bracket. These data indicate that failure to fit the size of loan to the farm rather than dollars per acre loaned was the cause of foreclosure.

Study of the loan-appraisal ratio indicates loans up to at least 75 per cent of normal agricultural values the level of those in the 1942-46 period were justified in all three areas. With this experience and with better knowledge of forbearance treatments even a larger percentage of the normal agricultural value probably could be loaned without excessive risk, particularly in Areas I and II, provided conditions during the loan repayment period were at least as good as in the two decades between World Wars I and II. The conclusion of the study by the Bureau of Agricultural Economics relating to the long-range outlook for American agriculture was that "the prospect for American agriculture over the next quarter century is relatively good." This BAE study indicates farm income in the next two decades probably will be at least as good, if not better, than during the inter-war period.

THE IOWA SCHOOL LUNCH PROGRAM: A THEORETICAL AND QUANTITATIVE ANALYSIS ¹

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The first student lunch projects were sponsored by individuals interested in humanitarian activities. In the United States, public interest in school feeding programs did not develop until the last decade of the nineteenth century, and then primarily in the largest cities. When the "great depression" focused public attention upon the problem of the co-existence of surplus commodities and hungry children, the Surplus Commodity Administration established a system under which locally sponsored projects might benefit by participation in the newly created Federal School Lunch Program. From the beginning, the primary objective was the development of an outlet for surplus commodities. However, the importance of dietary balance, achieved through planned supplementation gradually became recognized. In 1948 the legal provisions, which required that all participating schools maintain their normal amounts of purchase in addition to their surplus food grants, were no longer rigorously enforced.

The present study was undertaken to examine the compatibility of the nutritional balance and market support objectives, and to consider certain consequent welfare implications of the program. In connection with the objective of nutritional adequacy, the study was extended to examine the price mechanism as a means of effectively directing student food selections.

In order to determine the market support given by the program it was necessary to know the amounts purchased by the schools and by homes to serve the same number of lunches. The purchase records of schools were analyzed in terms of a theoretical model constructed to represent the schools' demand function. For the purpose of estimation this theoretical form of the function was simplified to include only those quantitative variables which were found to be statistically significant.

In the case of butter,² commodity (1), the equation was: $Q_{1(46-48)} = Q_1(P_{1(46-48)}, S_{(46-48)})$. $Q_{1(46-48)}$ equalled the amount purchased during the school years 1946-48. P_1 symbolized the price of the commodity during this period, and $S_{(46-48)}$ represented the number of lunches served for these two school years.

¹ Doctoral thesis number 1018, submitted December 13, 1949.

² In the case of celery, commodity (2), and poultry, commodity (3), their respective equations were $Q_{2(46-48)} = Q_2(P_{2(46-48)}, S_{(46-48)})$ and $Q_{3(46-48)} = Q_3(P_{3(46-48)}, S_{(46-48)})$.

For all other commodities, commodity (4), to and through commodity (n), the following equation³ was adopted: $Q_{4(46-48)} = Q_4(S_{(46-48)})$. In these equations the symbol, $Q_{4(46-48)}$, represented the amount purchased during the years 1946-48, and $S_{(46-48)}$, the number of lunches served for this period.

The home purchases were determined by utilizing two random samples, one of student lunch diaries in communities where there was no lunch program conducted by the school, and one of corresponding diaries for week-end and vacation periods in communities where the school operated a lunch program. In the comparison of the diaries of the two samples it was determined that they were from the same parent population.

The quantitative difference between the school and home purchases for the same number of lunches revealed the extent of support furnished by the school program for the lunch meal. This study revealed that the program positively supported certain items such as milk⁴ and many canned and fresh vegetables. However, the school program did not give positive lunch support⁵ to such commodities as beef and pork.

This investigation showed that the Iowa School Lunch Program contributed to the inflexibility of resource allocation by supporting the prices of such surplus items as potatoes, while at the same time it contributed to the flexibility of resource allocation, by stimulating the purchase of vegetables deficient in the student diaries and not in general surplus upon the market. Because the program positively supported more items of the second category, its current tendency has been to direct resource allocation into patterns more consistent with nutritional recommendations. An experimental design was developed to test the effectiveness of the price mechanism as a means of directing student food selections. The results showed that the average elasticities of demand for beets, string beans and white cake, between 2 and 5 cents, respectively were: -0.1, -0.47, and -2.5. This meant that with a 1 per cent change in price, there would be a corresponding change in quantity of 0.1 per cent, 0.47 per cent, and 2.5 per cent.

These results mean that the price mechanism may be used effectively to curtail the consumption of white cake. However, in the case of beets the price mechanism is not effective. In the case of string beans the price mechanism might possibly assist other directive techniques in ob-

³ Thus, for commodity (5), and commodity (n) the equation would be: $Q_{5(46-48)} = Q_5(S_{(46-48)})$, and $Q_{n(46-48)} = Q_n(S_{(46-48)})$.

⁴ Although the purchases of milk by the school program show a positive support for the lunch meal, it is not certain that there is a net support when total diaries are considered. The Iowa State College Department of Nutrition's present research indicates that the calcium intake of Iowa students in both groups, home and school lunch, is equal. This suggests that the home lunch students compensate for their lunch milk deficiency at some other time during the day, perhaps as an afternoon "snack."

⁵ The student total dietary protein intake for both of these groups was approximately equal. Until the source of the compensating protein intake of the school lunch group is determined, no conclusion may be drawn concerning the school lunch program's effect upon the student patrons' total meat consumption.

taining an increase of student purchases. However, because the regression coefficient of string beans was not significant, the data appear to be too fragmentary to support any definite hypothesis.

This analysis of the Iowa School Lunch Program showed that the nutritional and support objectives were compatible only coincidentally. If the grants of surplus food are used to complete the dietary patterns suggested by the nutrition experts the two objectives become compatible. If the program only serves as a convenient means of surplus disposal, regardless of the nutritional consequences, the two objectives frequently are in conflict. Recent policies indicate that in the future there may be greater effort exerted to assure the compatibility between these objectives.

If the present nutrition education programs succeed in changing the consumption patterns of many students, and thus of their future children, certain resource allocation problems will develop. Future research concerning the implications of nutrition education and its relationship to resource allocation appears to offer a promising field of endeavor.

ALLOCATION OF FARM RESOURCES FOR ECONOMIC PRODUCTION AND UTILIZATION OF PASTURE IN THE BLUEGRASS REGION OF KENTUCKY¹

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The production and utilization of pasture forage is a consideration in nearly every major production process on the farm firm in the Bluegrass Region of Kentucky on account of its close relationship to production of other crops and the livestock program. The core of the economic problem of pasture forage output is uncertainty of supply during the pasture production period; however, the economic level of output, and allocation of resources to pasture production and utilization are also important aspects. Much research has been done in these fields, but the task of fitting pasture production into the organizational structure of the individual farm is difficult, resulting in conflicting hypotheses and conclusions.

The objectives of this study were: (1) to clarify the economic relationships of production and utilization of pasture forage, especially as they relate to the Bluegrass Region; (2) to formulate hypotheses and to suggest appropriate tools of economic analysis for solutions to pasture problems; (3) to test hypotheses whenever primary or secondary data were available.

The method of study was to assume the role of an entrepreneur and to seek facts by deductive analysis of the problematic situations. The construction of economic models and formulation of hypotheses were attained by: (1) evaluating the historical development of the grazing system, the manner in which farmers met critical situations (panics, wars, drouths, inflation, etc.), and the ways that farmers improved their knowledge and farming methods; (2) appraising the economic limits of control over variability in supply of summer forage; (3) comparing the effects of substituting pasture forage for harvested forage on input combinations, economic returns, and scale of enterprises; and (4) integrating pasture in the farm plan to maximize the stream of net revenues to the firm.

To facilitate the analysis, the problem was delineated into four major areas of study: (1) the development of agriculture and the grazing system in the Bluegrass Region, (2) the production of pasture forage, (3) the utilization of pasture forage, (4) the over-all production plan.

The Bluegrass Region is circular in shape and includes an area of

¹Doctoral thesis number 1072, submitted June 6, 1950.

about 8,000 square miles. It is composed of three distinct subregions: the Inner-Bluegrass, the Intermediate-Bluegrass, and the Outer-Bluegrass. Each of the subregions is productive but the Inner-Bluegrass is most superior in all respects. In 1944, the gross cash income per acre in the Inner-Bluegrass was exceeded by only two counties in Illinois and one in Ohio among all of the counties in Ohio, Indiana, Illinois, and Kentucky. In that same year tobacco, which was grown on 6 per cent of the farm land, provided 73 per cent of the cash income. Sixty-two per cent of the land was devoted to pasture which in comparison to other areas of similar productivity was under-utilized.

Bluegrass, at the time of settlement (1775), was not a part of the natural vegetation that covered the land, as trees and canebrakes were the predominant growth. It spread rapidly after its introduction (apparently prior to 1775), as it was reported to cover the uncultivated open land by 1820. By 1830, writers were liberal in their praise of bluegrass and the grazing system of agriculture.

Virginia's method of apportioning the land to the early settlers by grants of 400 acres to families that settled before 1778, by grants for service in the Revolutionary War, and by treasury warrants made possible the formation of large estates. The combination of large estates, the Virginia heritage of the settlers, the rich soil, and the demand for staple commodities by the Cotton South provided an ideal condition for the development of slavery in the region.

A trip by General Wilkinson to New Orleans by way of the Kentucky, Ohio, and Mississippi Rivers in 1787 to sell Bluegrass-grown produce (mainly tobacco), marked the opening of an outlet for surplus farm commodities. The subsequent development of cotton farming, slavery, and large plantations in the South created a great demand for pork and pork products, hemp and hempen goods, flour, tobacco, beef, horses, and mules. Production of these products in a varying degree provided the most important sources of income from 1787 to the Civil War. Before 1820 the main emphasis was on cash crops, but after the panic of 1819 the system of farming changed to one of major dependence on livestock and livestock products. This system, except for several years prior to 1850, prevailed up to the Civil War and on a smaller scale afterward to 1890.

Livestock production (horses, mules, hogs, and beef cattle) from corn and bluegrass was the basis of the highly praised grazing system. The features of the grazing system were: (1) the seeding of the woodlands in grass, (2) the reliance on bluegrass pastures for 10 months of grazing (accomplished by under-utilizing pasture during the growing months), and (3) the feeding of corn fodder (ears attached) to the livestock during the remaining months. Hogs fitted into the grazing system by obtaining much of their sustenance from following fattening cattle, from red clover pastures, from gleaning the small grain fields after harvest, and from hogging down corn from the standing stalk. Cattle were fed to weights of 1,800 to 2,000 pounds and hogs to 400 or 500

pounds. Most of the cattle, horses, mules, and a large proportion of the hogs were driven to southern and eastern markets.

The peak of prosperity during the nineteenth century was probably reached in the 1830's. During this period farming methods were greatly improved and numbers of livestock on farms exceeded any other period of the century. In fact, the numbers of livestock were greater than they are at the present date. Coinciding with the development of the grazing system came the interest in breeding of "blooded" livestock such as the Shorthorn breed of cattle, the Thoroughbred and the American Saddle horse, and the mule. These efforts made an imprint on the respective types of livestock in the United States that cannot be discounted by the most severe critics.

The greatest setback to agricultural development experienced by Bluegrass farmers occurred in the decade following the Civil War when drastic reduction in all forms of agricultural production took place. However, the discovery of White Burley tobacco in 1868 and the high prices paid for it caused the Bluegrass farmers to quickly change their systems of farming, and by 1880 tobacco was a major product. The growing of tobacco as a major source of income strengthened the place of grass in the economy by making possible the cultivation of a smaller acreage in grain crops.

The historical study emphasized several pertinent points: (1) The external forces beyond the control of farmers were the greatest influence on changes in farming systems. (2) Livestock production was expanded during the periods of rising prices and prosperity of 1812-1816, 1825-1837, and 1850-1860. In contrast, hemp production increased in relative importance following the severe price declines of 1819, 1837, and 1860. (3) Many of the ideas and concepts that are considered a part of modern science were expressed with much thoroughness a century ago.

The emphasis changes now to analysis of the current problems of pasture production. However, we should note that the historical analysis of the problem provided significant information to explain the functioning of the Bluegrass economy under different environments.

The ideal pasture production program for a given farm firm was described as one that: (1) provided a maximum stream of pasture forage output with a minimum variation in supply and nutritive value within and between pasture production periods, and (2) had an acreage in pasture in proportion to other crops such that the stream of net revenues to the firm was maximized.

Data from a 13-year project on utilization of bluegrass pastures at Kentucky showed that the variation in output of pasturage forage was greater within periods than between periods. Analysis of variance of pasture yields indicated that the difference between monthly outputs was highly significant at the .01 level (F test), while the difference between the periods was not significant at the .05 level. Because of the marked variation within periods, and the needs of a livestock program for a stable supply, measures for reducing within-period variation

and their costs are of prime importance to farming systems based on pasture.

Rains over a 30-year period at Lexington, Kentucky occurred on 26 per cent of the days in June with 90 per cent of them less than one inch. As the season progressed, the rains fell on a smaller percentage of the days but tended to be larger. Drouths longer than 16 days occurred 4 out of 10 years in September and October and 2 out of 10 in July and August. A drouth between 26 and 35 days occurred once in 10 years in October, and 1 longer than 36 days occurred once in 16 years in September and part of October.

Experiments showed that output-increasing practices affected the dispersion of probable yield of pasture forage in a varying manner. In general, except for the addition of legumes in the plant mixture and irrigation, they reduced variation in good years, but tended to increase variation between good and poor years because of the impetus given to production in good years. Control of variation in supply of summer forage was most likely to result from a combination of the following methods: (1) choice of plant mixtures, (2) management practices, (3) storage of surplus production from one period for use in another, and (4) control of soil water supply.

The costs of reducing variation in supply of summer forage are of two types: (1) sacrifice of output by producing summer forage when the production per acre would be greater if spring forage were grown; and (2) cash outlay for certain arrangements for reducing the dispersion of probable supply. With respect to the first, the alternative of harvesting and storing the surplus of the spring production for summer consumption will be profitable if the cost is less than the value of the sacrifice in output. Cash costs for reducing seasonal variation can be added until the marginal cost of a unit reduction in variability is equal to its value. The value of a unit reduction in variability depends upon the relative importance of pasture to the main income-producing enterprises of the firm.

The output of pasture forage from a given acreage may be considered as a function of: (1) weather conditions, (2) topography, (3) soil type, (4) inputs of soil nutrients, (5) the plant population, and (6) grazing management. When all variables except plant population inputs (X_1) and inputs of soil nutrients (X_2) are considered as fixed, comparison of production surfaces for good and poor years will probably show the scale line closer to the X_2 axis in good years and closer to the X_1 axis in the poor years, indicating that in good years pasture production is relatively responsive to X_2 inputs and in poor years relatively more responsive to X_1 inputs. Also, for the good years the production surface will probably show greater complementarity between X_1 and X_2 . Output in the poor years will be maximized and variation between good and poor years minimized when the input combinations are chosen from the product contours of the good-year surface that produces the greatest output in the poor years.

Pasture forage in the previous discussion was considered as a

produce, now it is to be considered a factor in the production of livestock. Its value productivity, for any day, as a substitute in the feed rations of livestock, depends upon four classes of variables: (1) the types of livestock and their products, (2) the level of feeding and proportion of total feed supplied by pasture forage, (3) the quality of pasture forage, and (4) the prices for the factors and products. Because the entrepreneur wants to maximize the value productivity throughout the pasture production period, he will equalize the value productivity of one day with subsequent days.

Livestock output as a product is shown by the production function:

$$Y = f(X_1, X_2, X_3, X_4, X_5)$$

where Y is the livestock product: X_1 is grain; X_2 is protein feed; X_3 is harvested forage; X_4 is pasture forage; and X_5 is equal to all production factors (labor, capital, etc.). Pasture forage, X_4 , is a technical substitute for any one or all of X_1 , X_2 , and X_3 . It is considered as a perfect substitute (near) for harvested forage but complementary to concentrates. When pasture forage is fed, the marginal rates of substitution of grain for protein are likely to be greater than when harvested forage is fed. At the same price ratios for grain and protein feeds, the entrepreneur would feed proportionally less protein feeds to livestock on pasture than when on harvested forage. When protein feeds and grains are considered together as concentrates, the marginal rates of substitution of pasture forage for concentrates are probably greater than the rates of substitution of harvested forage for concentrates. If true, the optimum scale of the livestock enterprise will be greater when pasture forage is fed even though the prices of the factors are the same.

If the marginal value productivity of the same resources employed in another use is considerably greater than for pasture and if the "other use" can be employed without limit, the value of a unit reduction in variability of summer forage supply will be small. In this case, the entrepreneur may choose types of livestock that have demands for pasturage similar to the average pasture production pattern. Faced with a drouth and without appreciable flexibility arrangements, such an entrepreneur will probably resort to one or more of the following methods to minimize losses: (1) feed the reserve harvested forages intended for winter consumption, (2) sell the livestock, (3) effect a major change in the production equipment or storage facilities, (4) permit the livestock to use their reserve of body weight.

Pasture utilization for dairy cows differs from meat-producing livestock in that the production of each day is measured and weighed. Deciding the economical rate of daily output over the production period for a class of livestock producing a continuous flow of product is fundamental. The most important factors that cause variation in daily output are: (1) level of feed intake, (2) size of cows, (3) stage in lactation, and (4) inherent production. Research studies indicated that the principle of diminishing returns holds as the level of daily feed intake is increased,

making possible the determination of optimum daily output from the scale line once the technical rates of substitution (forage and concentrates) for successive outputs and the prices of factors and the product are known. The model used to describe the relationship showed that the ratio of concentrates to forage increased with larger daily outputs, and as larger daily outputs are realized the marginal rates of substitution of forage for concentrates diminish.

The problems of utilization of pasture forage by feeder cattle are similar to dairy production in that the cattle are at all times producing a daily output, and the entrepreneur desires to maximize the sum of the daily net revenues. They differ from dairy cattle in that the daily output is not measured or sold until some future date. Also the rate of daily output has considerable influence on the market grade at selling time. The value of a day's output at the beginning of the pasture production period is the price at which the entrepreneur will sell the cattle rather than feed them to a later market date. To him, each rate of daily gain will have a different value. The optimum daily output and the proportions to feed can be determined from the scale line as described for dairy cattle. If the cost of the factors were expected to change relative to the value of the produce the optimum daily rate of gain may be expected to vary over the period. Experiments conducted at Illinois to determine feed requirements to raise feeder cattle to higher market grades showed that daily gains from feeding grain-on-grass were as good as feeding in the drylot, and pasture, in addition to substituting for all the hay, replaced 22 to 33 per cent of concentrates.

To analyze the organization of the farm firm when uncertainty is a feature of importance to the over-all production plan, classification of products or product combinations according to relative returns and expected variability of returns is necessary to understand the actions of entrepreneurs in choosing their enterprises. Four classes are apparent: (1) high returns over time and high variability of returns, (2) high returns and low variability, (3) low returns and high variability, and (4) low returns and low variability. The ideal combination for most entrepreneurs is high returns and low variability, and the untenable combination is low returns and high variability. Logically, the condition of high returns and low variability cannot persist over time unless monopolistic elements are present. In this case, the relative returns may diminish because of the tendency to capitalize the security of returns into the structure of the firm, or as in the case of tobacco, the base allotment.

In general, a high proportion of variable costs relative to fixed is associated with high returns and high variability, and low variable costs relative to fixed are associated with low relative returns and low variability. Bluegrass pastures, without control over the variability of summer forage supply, are typical of the case of low variable costs relative to fixed. Consequently, the selection of livestock that have production patterns corresponding to bluegrass production pattern, and have costs which are largely fixed (sheep and beef cow herds), makes a natural

combination with tobacco. Before governmental control of acreage, tobacco was in the class of high returns and high variability. A loss in tobacco production was offset, in part, by income from an enterprise or enterprises of low variable costs. On the other hand, entrepreneurs with confidence in their ability to outguess their fellow producers and influence the skewness of the distribution of costs and product prices chose a combination of two enterprises of high returns and high variability. Tobacco and heavy feeder cattle were an example of this condition. Wind-fall profits when they occurred, were large.

Indivisibility of resources is a significant obstacle to firms in pasture-producing areas because stabilizing the supply of summer forage usually means investment in forage harvesting equipment, storage structures, and possibly irrigation. Small firms or firms not requiring power equipment for other types of crop production experience difficulty in providing sufficient employment to include such investment in the long-run plan. The frequent result is under-utilization of the pasture forage, or higher costs.

Without control over variability of the supply of summer forage the firm may be forced to select types of livestock that can subsist for considerable periods of time on maintenance rations, or it may under-utilize the pasture forage during the growing period to leave a residue on the land for consumption in a deficit period, or it may do both. Also, the segment of the farm business associated with pasture production will probably have a low elasticity of production because of the tendency toward inflexibility in the ratio of fixed to variable costs. If such a firm is wholly dependent upon pasture for its sources of income it will tend to be a type of business in the class of low returns and low variability.

The ideal pasture forage program is not beyond realization by many firms. Investment in stabilizing the summer supply of forage will be profitable if the pasture-produced secondary product results in a greater value productivity for labor and capital than a competing enterprise. On the other hand, when the marginal value productivity of competitive resources is higher for primary production, and can be employed without limit, stabilizing the supply of summer forage may not be economic. The firm exercising the control will have more flexible arrangements for meeting unfavorable events than the noncontrol firm and it will probably have types of livestock which are at all times producing a product.

STORAGE OF MEAT IN CARBON DIOXIDE ATMOSPHERES AT TEMPERATURES ABOVE FREEZING ¹

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The present work was intended to show the effectiveness of atmospheres containing carbon dioxide in increasing the shelf life of cut-up chicken and frankfurters and to determine the most favorable conditions for its employment. Other investigators have demonstrated that carbon dioxide retards growth of some microorganisms and prolongs the storage life of certain meat products. Since deterioration of chicken or frankfurters is primarily due to microorganisms proliferating on the meat surface, this investigation was largely microbiological in nature.

In one series of experiments, chicken samples were stored in sealed containers until spoiled. Solution of carbon dioxide in the meat resulted in decreased CO₂ concentrations and reduced pressures within the storage containers. A diminution in oxygen level also was noted; after several days' conditions were essentially anaerobic. At low oxygen tension, CO₂ was of less value in prolonging keeping time and spoilage was putrefactive in nature. The predominant types of bacteria were shown to differ from the kinds commonly producing slime on chicken stored in air.

In a major portion of the work, carbon dioxide concentrations were maintained at a nearly constant level during storage either by continuous flow of gas through the containers or by periodic replacement of atmospheres. Keeping times for cut-up chicken were determined from the time required for: (1) a definite off-odor to be detected; (2) a specified increase in CO₂ to be observed in storage containers; (3) numbers of bacteria to reach a value of 2×10^8 per square centimeter of chicken surface. Good agreement was obtained upon comparing the three methods.

In general, storage life increased as higher levels of carbon dioxide were employed. Large variations in keeping time were encountered with pieces of chicken stored under the same conditions. Shelf life under given storage conditions was found to depend on initial bacteriological quality. The influence of CO₂ was measured by the storage index (ratio of keeping time in CO₂ to keeping time in air); storage index also varied with initial bacterial load. For example, storage indices obtained for chicken backs stored at 40°F. (4.4°C.) with 15 per cent CO₂ varied from 1.42 to 2.25. A linear relationship between storage index and carbon dioxide concentration was observed.

¹Doctoral thesis number 1037, submitted March 13, 1950.

Growth of slime forming bacteria on the surface of cut-up chicken was followed by means of plate counts. Average generation times of the proliferating bacteria were obtained for several conditions of storage. Average generation time appeared to be a logarithmic function of CO_2 concentration within the range 0 to 25 per cent. Increasing CO_2 level also tended to prolong lag time although no definite relationship could be demonstrated.

Reduction of storage temperature markedly improved keeping time of cut-up poultry. Temperatures of less than 50°F . (10°C .) were considered essential for satisfactory storage life with or without carbon dioxide storage. The effectiveness of CO_2 in retarding bacterial growth was enhanced by a decrease in temperature. In the range 32° to 50°F . (0 to 10°C .), the relationship between rate of bacterial growth and temperature conformed rather well with the Arrhenius-van't Hoff equation for storage in air and in atmospheres containing 15 and 25 per cent CO_2 .

Carbon dioxide concentrations of 80 and 96 per cent caused serious discoloration of chicken meat within 2 days. Occasionally, some loss of "bloom" was noted with levels of 15 and 25 per cent CO_2 when keeping times were unusually long.

Slime producing bacteria were predominantly members of the genus *Pseudomonas*. Another important group was similar to *Alcaligenes viscosus*. Temperature (32° to 50°F .) and carbon dioxide concentration (0 to 25 per cent) did not affect the types of bacteria isolated from two lots of stored chicken. Some differences in type were noted between lots.

Storage life of frankfurters was improved by an increase in carbon dioxide concentration or a reduction of temperature. No additional benefit resulted when CO_2 levels above 50 per cent were employed. Reduction of temperature again increased the effectiveness of CO_2 . Rancidity developed before microbial spoilage was observed when a storage temperature of 30°F . (-1.1°C .) was used in conjunction with atmospheres containing 15 and 50 per cent CO_2 .

Relative growth rates of the various kinds of microorganisms causing deterioration of frankfurters varied with the CO_2 level employed. Organisms primarily responsible for spoilage changed from molds to yeasts and micrococci and then to lactobacilli with increasing carbon dioxide concentration. Rates of growth of molds, yeasts, and micrococci were progressively retarded as CO_2 level was elevated; CO_2 had much less influence on the growth rate of lactobacilli. At CO_2 levels of 50 per cent and above, the predominant flora consisted almost entirely of lactobacilli.

In the case of stored frankfurters, visible evidences of microbial deterioration ordinarily were noted before definite changes in flavor or pH could be detected. With CO_2 levels of 50 per cent and higher, spoilage first appeared as a watery slime composed of lactobacilli; end points could not be established with certainty. Measurement of pH

showed promise as a means of determining end points when high CO₂ percentages were employed.

Neither color nor flavor of frankfurters was affected adversely by carbon dioxide.

Use of relative humidities below 95 per cent caused pronounced dehydration of frankfurters without noticeably prolonging keeping time. Reduction of relative humidity to 95 per cent did not appreciably retard the growth of molds, yeasts, and micrococci; however, no increase in numbers of lactobacilli was noted at that humidity.

L.S.A.T. cellophane did not maintain a carbon dioxide atmosphere around packaged frankfurters.

A holding period in atmospheres containing CO₂ followed by storage in air resulted in longer keeping times for chicken and frankfurter samples than did storage in air only.

INFLUENCE OF pH ON PROLIFERATION OF *STREPTOCOCCUS LACTIS* BACTERIOPHAGE¹

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Five strains of lactic streptococcus bacteriophage were studied over a wide pH range to determine the influence of pH on bacteriophage proliferation. Bacteriophage strains of as widely different characteristics as possible were selected for this study. Litmus milk, adjusted to the desired pH with 1N NaOH or 10 per cent lactic acid, was used as the basal medium for all studies at pH 5.2 or above and a V-8 juice-peptone-peptonized milk broth was used for studies at pH levels below 5.2. The pH of the substratum was maintained during the trials by the periodical addition of 1N NaOH. All organism counts were made by the plate method, using TGEM agar. Bacteriophage numbers were determined as the most probable number by a three-tube limiting dilution technique and all results were at an incubation temperature of 32°C.

The optimum pH for proliferation of the streptococcal bacteriophage was on the acid side of neutrality, at about pH 6.5. However, considerable proliferation occurs over a pH range from 5.4 to 7.5, with some strains extending beyond this range. The maximum and minimum pH at which bacteriophage proliferation occurred varied for each strain studied. These limits extended from a low of pH 4.8 to a high of pH 9.4.

The bacteriophage strain F24 multiplied wherever there was positive growth of its homologous organism and the pH influenced only the rate of proliferation. At pH 4.8 proliferation of the bacteriophage occurred after an extended lag phase and the rate was very slow. The host organism W2 followed a similar pattern at low pH levels. Bacteriophage F24 proliferated at pH 9.4 but only after a long lag phase. Mass lysis occurred with W2-F24 combination at pH 5.0 and pH 7.6 with indications that mass lysis may occur at pH levels as high as 8.0 and as low as 4.9 or below under proper conditions.

The range of pH for proliferation of F55 was quite narrow. The minimum pH was 5.2 while at pH 7.6 proliferation was greatly retarded. There was a very distinct difference in rate of proliferation of F55 at pH 7.5 and pH 7.6. At pH 7.6 proliferation of the bacteriophage occurred after a long lag phase but very slowly as compared to pH 6.5 or even pH 7.5. The difference in growth of the *S. cremoris* 573 at pH 7.5 and pH 7.6 was not significant.

With strain F56 very little proliferation occurred below pH 5.0 whereas the host organism 712 multiplied very rapidly at pH levels as low

¹ Doctoral thesis number 1057, submitted June 1, 1950.

as 4.7. Proliferation of F56 occurred after a prolonged lag phase at pH 8.4 but the *S. lactis* 712 did not show the same sensitivity to the alkaline reactions.

Very little proliferation of the bacteriophage or the organism occurred at pH 5.0 with the H1,1-F4 combination. The growth of H1,1 at pH 9.4 was erratic and the same was true of proliferation of the bacteriophage. If the organism became adapted to the highly alkaline conditions and definitely increased, the bacteriophage proliferation was very rapid.

In general bacteriophage proliferation failed to occur at pH levels where organism growth was inhibited. However, in one trial at pH 8.0 with the 122,1-F43 combination it appeared that the cells were in the right physiological condition to support bacteriophage proliferation without an increase in organism number as determined by the plate count. With three combinations, 122,1-F43, 712-F56 and 573-F55, growth of this organism occurred beyond the pH ranges of bacteriophage proliferation.

There was no correlation between the type of organism and the bacteriophage response to pH. Organisms W2 and 712 are classified as *S. lactis*, while H1, 1, 573 and 122,1 are *S. cremoris*. The "cremoris type" usually are considered more fastidious and more sensitive to adverse conditions and one might expect a similar situation in the homologous bacteriophage, but this was not found to be true. With the H1,1-F4 combination the bacteriophage was able to multiply at pH 9.4 and with the 122,1-F43 combination, F43 multiplied at pH 5.0, while proliferation of F56 on *S. lactis* 712 was greatly retarded at pH levels of 5.0 and 8.4.

The extremes in response to pH are probably represented in the five strains of bacteriophage studied, since the proliferation of F24 occurred from pH 4.8 to pH 9.4, which approximates the limits of growth of the *S. lactis* species. F55 could easily represent the other extreme, since its proliferation was confined to such a narrow range of pH (5.2 to 7.6). The probability of obtaining strains vastly different in their response to pH does not seem likely.

In the dairy industry the occasional failure of a seemingly normal culture may be explainable in part by the influence of pH. The organisms may lower the pH so rapidly that mass lysis does not occur, despite the build-up of relatively high concentrations of bacteriophage. Under these conditions the culture would coagulate and appear normal, but when used as inoculum in milk with a pH near the optimum for bacteriophage proliferation, lysis of the susceptible cells would occur in a few hours.

TORSION AND FLEXURE OF COMPOSITE SECTIONS¹

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The Saint Venant torsion and flexure problem is formulated for beams whose sections are composed of different materials. Two types of sections are considered, namely:

- A. Sections composed of different isotropic materials,
- B. Sections of which a portion is isotropic and the remainder orthotropic.

General methods are developed for handling the torsion and flexure problem for sections involving only two different materials. These methods may be easily extended to handle the case of three or more materials.

A beam of length l is taken with its axis in the z direction. The end $z = 0$ is fixed and the end $z = l$ is loaded. This load may be resolved into components parallel to the x and y axis producing only bending and a twisting couple producing pure torsion, the effects of which may be superposed. The sections considered have an outer boundary C_1 , which is free from traction, and a common boundary, C_2 . The boundary C_2 divides the section into two portions possessing different elastic properties. The boundary conditions amount to no normal shear along C_1 , and continuous displacement and tractions across C_2 .

The equations for stresses and displacements for the torsion problem are taken as:

$$\tau_{xz} = a\mu_i \left(\frac{\partial \phi_i}{\partial x} - y \right),$$

(1)

$$\tau_{yz} = a\mu_i \left(\frac{\partial \phi_i}{\partial y} + x \right),$$

$$u = -ayz, v = axz, w = a\phi_i(xy) \quad (i = 1, 2)$$

where the value of i depends upon the portion of the section considered. If the section is composed of two different isotropic materials the problem becomes one of determining two functions, ϕ_1 and ϕ_2 , satisfying the boundary conditions and the differential equations:

$$\nabla^2 \phi_1 = 0, \text{ for one portion}$$

(2)

$$\nabla^2 \phi_2 = 0, \text{ for the second portion.}$$

¹ Doctoral thesis number 1048, submitted May 19, 1950.

In this case the torsional rigidity, D , is found from the equation:

$$(3) \quad D = 2\mu_1 \iint_{A_1} \phi_1 dx dy + 2\mu_2 \iint_{A_2} \phi_2 dx dy,$$

where ϕ_i is defined as $\psi_i - \frac{1}{2}(x^2 + y^2)$ and ψ_i is the harmonic conjugate of ϕ_i .

If one portion of the section is isotropic and the remaining portion is orthotropic, two functions, ϕ_0 and ϕ_1 , have to be determined so as to satisfy the boundary conditions and the differential equations:

$$(4) \quad \begin{aligned} \nabla^2 \phi_0 &= 0, \text{ for the isotropic portion,} \\ \mu_1 \frac{\partial^2 \phi_1}{\partial x^2} + \mu_2 \frac{\partial^2 \phi_1}{\partial y^2} &= 0, \text{ for the orthotropic portion.} \end{aligned}$$

In equation (4) μ_1 is the shear modulus in the x direction and μ_2 is the shear modulus in the y direction for the orthotropic portion of the section. For this type of composite section the torsional rigidity is given by:

$$(5) \quad D = 2\mu_0 \iint_{A_1} \phi_0 dx dy + 2\sqrt{\mu_1 \mu_2} \iint_{A_2} \psi_1 dx dy - \mu_2 I_{y^2} - \mu_1 I_{x^2}.$$

In equation (5) I_{x^2} and I_{y^2} are the moments of inertia with respect to the x and y axes respectively of the orthotropic portion of the section. The function ϕ_0 is defined in a manner similar to the ϕ_i in equation (3). The function ψ_1 is found from the equations:

$$(6) \quad \frac{\partial \psi_1}{\partial x} = \sqrt{\frac{\mu_1}{\mu_2}} \frac{\partial \phi_1}{\partial x}, \quad \frac{\partial \psi_1}{\partial y} = -\sqrt{\frac{\mu_2}{\mu_1}} \frac{\partial \phi_1}{\partial y}.$$

The flexure problem resolves itself into the determination of flexure functions, χ_i , satisfying the boundary conditions and the differential equations:

$$(7) \quad \begin{aligned} \nabla^2 \chi_i &= 0, \text{ over any isotropic region,} \\ \mu_1 \frac{\partial^2 \chi_i}{\partial x^2} + \mu_2 \frac{\partial^2 \chi_i}{\partial y^2} &= 0, \text{ over any orthotropic region.} \end{aligned}$$

The stresses and displacements are given in terms of ϕ_i and χ_i in the usual manner.

The concept of the center of elasticity is introduced in the solution of the flexure problem to get a distinction between geometric symmetry and elastic symmetry. It is shown that in the case of the composite section, loading of the member along a geometric axis of symmetry of

the section does not insure the absence of a twisting effect. The coordinates of the center of elasticity are found to be:

$$(8) \quad x_c = \frac{E_1 A_1 \bar{x}_1 + E_2 A_2 \bar{x}_2}{E_1 A_1 + E_2 A_2}, \quad y_c = \frac{E_1 A_1 \bar{y}_1 + E_2 A_2 \bar{y}_2}{E_1 A_1 + E_2 A_2}$$

where the E 's are the moduli of elasticity in the z direction of the respective portions of the section. \bar{x}_1 and \bar{x}_2 are the x coordinates of the geometric center of the respective portions of the cross section. The y coordinates of the geometric center are designated by \bar{y}_1 and \bar{y}_2 . If the Young's moduli in the z direction are identical for the two portions the center of elasticity will correspond to the centroid of the section.

Solutions are obtained for sections whose boundaries are concentric circles, similar ellipses, confocal ellipses, eccentric circles and rectangles. Numerical results comparing the torsional rigidities of a number of sections have been tabulated.

FACTORS AFFECTING THE ENZYMIC SYNTHESIS OF PEPTIDE BONDS¹

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The purpose of this investigation was the determination of the effects of experimental conditions on the synthesis of benzoylamino acid anilides from benzoylamino acids and aniline as catalyzed by papain in citrate buffer solution. The data thus obtained might be basic to the synthesis of peptide bonds in general. The factors have included pH, substitution in the side chain of phenylalanine, citrate buffer concentration, benzoylamino acid: aniline ratio, volume of buffer employed for the reaction, and time of reaction. Rates of formation were studied for the anilides of the benzoyl derivatives of glycine, alanine, valine, and leucine. The extent of hydrolysis of the amides of glycine, valine, and leucine were determined.

Significant differences were found in the effect of pH on the papain-catalyzed synthesis of benzoylamino acid anilides. The optimum pH for the synthesis of the anilides of benzoylglycine and benzoylalanine was 5.0–5.5. The optimum pH for the syntheses of the anilides of the benzoyl derivatives of leucine, phenylalanine, tyrosine, and *p*-methoxyphenylalanine was between 6.0 and 6.5.

At the traditional optimum pH for papain, pH 5.0, the differences in reactivity between benzoylphenylalanine and N-benzoyltyrosine amounted to a virtual specificity. No demonstrable yield of N-benzoyltyrosinanilide was obtained at this pH but good yields of benzoylphenylalaninanilide resulted. When citrate buffers at higher pH values were used, good yields of benzoyltyrosinanilide as well as benzoylphenylalaninanilide were obtained. These results demonstrated that specificity requirements of proteolytic enzymes are determined to an appreciable extent by the hydrogen ion concentration.

The use of 1.0 *M* citrate buffer in place of the customarily used 0.1 *M* citrate buffer resulted in increased yields of benzoylamino acid anilides. The increase in yield was typically three- or four-fold, for the experimental conditions used in these studies.

The benzoylamino acid anilides were obtained in increased yield when the ratio of aniline to benzoylamino acid was increased.

The volume of buffer employed in the reaction affected the yields of anilides obtained. When the concentration of all the reactants was increased by diminishing the volume of citrate buffer while holding the amounts of benzoylamino acid, aniline, papain, and cysteine hydro-

¹ Doctoral thesis number 993, submitted August 27, 1949.

chloride constant, the amount of anilide obtained in a 3-day period was increased.

For the benzoylamino acids studied, commercial papain catalyzed as well as a papain preparation which had received preliminary treatment. This treatment involved bubbling hydrogen sulfide through an aqueous solution of the commercial enzyme, filtering off any insoluble material and then precipitating the enzyme by adding methanol until a 60 per cent solution of methanol was obtained. For methods of resolution of racemic amino acids, enzymic syntheses, and the studies on the effect of experimental conditions on the syntheses of benzoylamino acid anilides, commercial papain is apparently as satisfactory as the treated preparations of the enzyme.

Six days' incubation resulted in only slightly greater yields of benzoylalaninanilide and benzoylphenylalaninanilide than did 3 days' incubation. The rate studies on the formation of the anilides of the benzoyl derivatives of glycine, alanine, valine, and leucine also indicated that equilibrium was not reached in 3 days.

It was shown from the rate studies on the formation of the anilides of the benzoyl derivatives of glycine, alanine, valine, and leucine that the rates of formation of the anilides increased in the order: valine, glycine, alanine, and leucine. Addition of solid anilides to the reaction mixtures at the beginning of the incubation period did not result in significant variations in yield. These "seeded" rate studies indicated that variations in yield were not due to supersaturation effects. On the basis of all of these rate studies it was concluded that the variations in yields of anilides were due to differences in rate of reaction of the benzoylamino acids rather than to differences in the physical properties of the anilides.

Benzoylleucinamide was hydrolyzed to a significantly greater extent than was benzoylvalinamide when the amides were incubated in citrate buffer in the presence of papain-cysteine. This greater reactivity of the leucine derivative in contrast to the valine derivative for this hydrolysis reaction indicated that in both hydrolysis as well as synthesis an enzymic preference existed for leucine derivatives.

In the absence of data for special enzymes for protein synthesis, and considering that the same proteolytic enzyme can catalyze both the hydrolysis and synthesis of peptide bonds under essentially similar conditions, the proteolytic enzymes deserve serious consideration as primary catalytic agents for protein synthesis. Since the enzymic preferences pointed out above exist for both hydrolysis and synthesis, and since these preferences have been shown to depend upon enzymatic behavior rather than on the physical properties of the anilides, the validity of the anilide syntheses as models of the biological synthesis of peptide bonds now becomes worthy of more serious consideration.

The effects of experimental conditions on the syntheses of benzoylamino acid anilides are pertinent to the development of methods of resolving racemic amino acids.

AN ECONOMIC ANALYSIS OF THE INDIANAPOLIS FLUID MILK MARKET ¹

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This study was undertaken to analyze the economic forces operative in the Indianapolis fluid milk market and to develop long-run pricing policies for the market. Data were obtained from market organizations for the years 1936 to 1949 and from interviews of a random sample of milk producers. Multiple regression and other statistical techniques were employed as tools of analysis.

Indianapolis is a major metropolitan center with nearly 550,000 residents. The milkshed extends about 50 miles from the city covering the central Indiana grain and livestock region in which dairying is a secondary source of farm income. About 4,300 milk producers deliver milk to Indianapolis of whom about two-thirds are grade A producers and 97 per cent are members of the Indianapolis Sales Association, the principal producers' bargaining association in the market. The marketing of all milk from producers to the eighteen dealers in the city is governed by a contract between them and the Sales Association. The actual work of negotiation is performed by a marketing committee of producers' and dealers' representatives. Milk is priced to dealers on a use-classification basis and market-wide uniform prices are paid producers. The Sales Association performs most of the functions of a market administrator's office in government regulated markets including the auditing of dealers' records and the computation of uniform prices to producers. Although this market structure offers producers and dealers opportunities for collusion at the expense of consumers, evidence of such action is lacking and both retail and farm prices of milk have been close to prices in other markets of comparable size. On the whole the system has been a flexible, workable, and relatively inexpensive means of bargaining between producers and dealers. Significant operating economies could be achieved, however, through merger of the four producers' cooperatives with the Sales Association.

On the basis of the micro-theory of consumer choice and of an analysis of the ways in which consumers use milk the hypothesis was formulated that per-capita milk consumption in Indianapolis is a function of its retail price, the retail price of evaporated milk, the retail prices of all foods (as a group), the retail prices of all consumer goods (as a group), disposable income per capita, and consumer preferences. Annual data of the Indianapolis market for the 1936-48 period were first

¹ Doctoral thesis number 1017, submitted December 13, 1949.

studied by graphic correlation procedures and the relationships among the variables were found to be linear in character. The multiple regression equation best describing the relationships between per-capita consumption and other variables was found to be:

$$\hat{X}_1 = 70.142 - 0.885 X_2 + 1.028 X_4 + 0.089 X_5 + 0.086 X_7$$

X_1 = the index of per-capita milk consumption (1936-40 = 100), X_2 = the index of the retail price of milk, X_4 = the index of the cost of living, X_5 = the index of per-capita disposable income, and X_7 = X_5 advanced one year (as a measure of past living standards). The coefficient of multiple correlation, $R_{1.2457} = 0.958$, is highly significant but with only a few degrees of freedom the standard error of estimate, $\hat{\sigma}_{1.2457} = 1.635$, is large and the 95 per cent confidence intervals of the coefficients of partial regression are so large as to include values opposite in sign to the sign of the coefficient. As a consequence, the statistical findings were not used as evidence to support the hypotheses previously set forth. However, on the basis of an economic analysis it appears that per capita consumption of milk and cream is affected negatively by changes in the retail price of milk. Other retail prices, namely, the prices of evaporated milk, all foods, and of all cost-of-living commodities appear to affect fluid milk consumption positively. Both current and past incomes appear to have similar effects also.

On the basis of essentially similar procedures it was found that the relationship between production per cow in Indiana during the 1924-41 period and other variables deemed likely to be related to it was best described by the equation:

$$\hat{V}_1 = 83.858 + 0.255 V_2 - 0.153 V_3 + 0.439 V_8$$

V_1 = the index of milk production per cow, Indiana (1936-40 = 100), V_2 = the index of the average Indiana farm price of whole milk, V_3 = the index of the cost of the Indiana dairy ration, and V_8 = time (in years). For this analysis Indiana data were used because Indianapolis data were not available. The value of $R_{1.238} = 0.914$ is highly significant and $\hat{\sigma}_{1.238} = 0.485$ is not large. The 95 per cent confidence limits of the regression coefficients were found to be narrow also. The farm price of milk and the costs of dairy feeds were the most important factors affecting production per cow. Gradual improvements in herd qualities seemed to be an important long-run factor. The number of cows on Indiana farms, by a similar analysis, was found to be related closely only to the long-time upward trend in cow numbers in this state. The prices of cows and farm wages were found to be positively related to both milk production rates and milk prices and appear to have been influenced by milk prices and other factors affecting farm incomes. The prices of hogs, annual average pasture conditions, and all Indiana farm prices were not closely associated with milk output rates. In

general, analysis of the interviews of the 109 sample Indianapolis milk producers supported the foregoing conclusions. Milk production rates and cow numbers change little in the short run as the result of changes in prices and other variables, and long-term plans appear to be the major factors underlying farmers' decisions relative to milk production rates and cow numbers. On the basis of both the survey of producers and analysis of Indianapolis market data it appears, however, that farmers are sensitive to changes in price relationships among markets. There are a large number of alternative outlets for milk available to farmers in the vicinity of Indianapolis and the degree of competition among these markets and with the Indianapolis market appears to be high.

The problem of seasonally uneven milk production was found to be both economic and physical in nature. Milk production costs are generally believed to be higher in the fall and winter months than in other seasons. Variations in the seasonal production patterns on Indianapolis milkshed farms were not closely related to such characteristics of the farm as the major source of income, the amount or grade of milk produced, or the tenure status of the operator. About 80 per cent of the Indianapolis milk producers deliver substantially more milk in the spring than in the fall giving as their principal reason for this production pattern the cheapness of feed from pastures. From 1936-48 Indianapolis grade A blend prices averaged 26 per cent higher in November and December than in May and June. This variation, it appears, must be increased if the market is to be able to induce a substantial shift in the average seasonal production pattern in the milkshed.

The price structure of the Indianapolis fluid milk market is complex and there is evidence that monopolistic forces are operative. However, the monopolistic powers of producers and dealers are significantly limited by the presence of alternative markets and potential supply sources within the milkshed. Accordingly, it is important that Indianapolis prices to producers be maintained at levels close to the average of prices paid producers in competing manufacturing milk markets subject to differences based on variations in milk quality and in seasonal delivery patterns of fluid and manufacturing markets. The Indianapolis Class II price formulae based on the average prices paid by five selected condenseries and upon the wholesale price of butter do much toward accomplishing this. The Class I price which is the Class II price plus a negotiated premium is a key factor, also, in the determination of grade A blend prices, retail prices, and the volumes of sales and receipts in the market, and it is important that the Class I differentials be set so as to promote equilibrium between sales and receipts of milk in the market. During the 1936-48 period in Indianapolis it was found that the relationship between Y_1 = annual Class I sales in per cent of grade A receipts and Y_2 = annual Class I prices in per cent of Class II prices was on the average described by:

$$\hat{Y}_1 = 156.66 - 0.61 Y_2, \text{ with } r_{12} = -0.83 \text{ a highly significant value.}$$

Since the seasonal index of Y_1 in December was found to be 123 and Indianapolis dealers usually must make supplemental purchases of milk whenever Class I sales exceed in any month 90 per cent of grade A receipts from local producers, the annual equivalent Class I utilization percentage is about 73 per cent ($90 \div 123 \times 100$). If $Y_2 = 135$ per cent is substituted in the above equation we find that Y_1 is about 74 per cent. Therefore, it appears that the annual Class I price in Indianapolis should be in the neighborhood of 135 per cent of the annual Class II price, permitting grade A blend prices to average about 26 per cent above ungraded blend prices under average conditions in the market.

From a somewhat similar analysis of market data it was estimated that the seasonal range of Class I prices should be in the neighborhood of 35 per cent higher in November and December than in May and June, assuming normal seasonal movements in the Class I utilization percentage and Class II prices. Blend prices under these conditions can be expected to average about 44 per cent higher in November and December than in May and June.

Alternative means of adjusting supplies of milk to consumer requirements were studied and three methods—the temporary Production Incentive Plan operative in the 1948–49 fall-winter seasons, seasonal supplemental purchases, and seasonal surplus disposal operations—were rejected as permanent marketing policies primarily on the basis of their high costs. Both supplemental purchases and disposal of surpluses, however, may be necessary at times as temporary measures to effect a balance between sales and receipts. The “take-off and pay-back” plan of seasonal pricing to producers was analyzed and found insufficiently superior to the seasonal pricing methods now employed in Indianapolis to justify recommending it as a permanent pricing plan for the market.

No other major pricing changes were recommended. Had they been followed in the past the serious surplus disposal and shortage problems would have been alleviated greatly in most years.

NITROGEN FERTILIZATION OF SMALL GRAIN AND ITS EFFECT ON COMPETITION WITH THE LEGUME-GRASS COMPANION CROP¹

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Failures in legume stands and reduction in yields are a serious problem in Iowa. Part of this failure of the seeding can be attributed to the increased competition between the legume and its oat companion crop following the use of relatively high rates of nitrogen fertilizer on oats. With the widespread use of improved, stiff-strawed oat varieties, nitrogen fertilizer has been used quite successfully to increase oat yields. In seventy experiments conducted in Iowa during the past 6 years, oats gave significant responses to nitrogen fertilizer in more than 90 per cent of the studies. The greatest yield increase per unit of nitrogen was obtained from the use of 20 pounds per acre, but the highest oat yields were commonly obtained from the use of 40 pounds of nitrogen or more, except in dry years. However, the yields of hay following nitrogen fertilization were significantly reduced in about 50 per cent of the experiments. This series of greenhouse and field studies was conducted in 1947, 1948, and 1949 to study the nature of the competition between fertilized oats and the legume-grass companion crop and to test methods of decreasing this competition.

It was found that yield reductions were not confined to any one legume or grass; however, red clover appeared to be affected more by the competition than alfalfa or sweet clover.

The critical competition, brought about by the increased oat vegetation following nitrogen fertilization, is primarily for sunlight and moisture. Competition for soil nutrients may play a minor part in stand and yield reductions of seedings. Added phosphorus often increases the stand and yield of the legumes, but does not eliminate the detrimental effect of nitrogen fertilizer applied to the previous oat crop.

Light intensities under fertilized oats may be 50 per cent lower than under unfertilized oats. In greenhouse and field studies with controlled light intensities, it was noted that at reduced light intensities legumes and grasses not only make less top growth, but their root development is even more drastically curtailed. Under severe shade roots of the young seedings may be confined to the upper 6 to 10 inches of soil, the plants are quite succulent and legume nodulations were reduced. These conditions cause legume seedlings to grow slowly and produce a shallow root system, and when competition for soil moisture

¹ Doctoral thesis number 1058, submitted June 1, 1950.

develops, they may not be able to obtain sufficient moisture from the surface soil to survive periods of limited rainfall. Surface soil moisture was found to be near the wilting percentage at oat harvest time in 1947 in Western Iowa. A combination of low light intensities, resulting in a reduction in root development of the seeding, and low soil moisture may be the cause of most of the failures in seeding stands and of low hay yields following the use of relatively high rates of nitrogen on oats.

Several methods of reducing the competition between companion crops were studied. Some of these methods were not effective, and others were not practical. The value of any of the methods will depend largely upon soil and climatic conditions of the area in which it is to be used.

Some preliminary experiments, not reported in this thesis, had indicated that reducing the rate of planting of the companion crop did not materially benefit the seeding, as thinly planted oats exhibit a great faculty for stooling when fertilized. This resulted in an almost uniform population regardless of the number of seeds originally planted. Non-fertilized oats or oats grown on soil of low productivity generally do not stool profusely, but under these conditions the competition from oats is probably not great at any rate of seeding.

Oat straw returned to the stubble after harvest as a protection for the young shade-grown seeding did not result in an increased yield of the hay the following year.

An addition of nitrogen to the legume-grass seeding after oat harvest also failed to improve hay yields the following year.

There was little difference in the value of the three oat varieties—Clinton, Marion, and C.I. 3846—as companion crops. These varieties were probably not sufficiently different in growth habits to cause any variations in the yield of the seedings. Other varieties that show greater differences in growth habits may make more desirable companion crops than the ones tested; however, such varieties are not commonly grown in Iowa at the present time.

Early removal of oats was not generally a satisfactory method of reducing crop competition, as the young seeding was probably injured by the frequent clipping. Cutting the companion crop relatively late for hay is sometimes beneficial to the seeding, but is not generally recommended since the oat grain yield is ordinarily more important than the oat hay.

Seedings on plots receiving delayed applications of nitrogen had somewhat better stands and yields than those on plots receiving added nitrogen at planting. Oat yields were not reduced on these plots if the nitrogen application was not delayed beyond the third week after plant emergence. The reduction in competition from the delayed applications of nitrogen as compared to applications made at seeding is probably due to the smaller vegetative response from the late application. Late application of nitrogen may also delay competition until after the seeding has an opportunity to become established.

Another method of reducing competition between companion crops without decreasing oat yields appears to be that of drilling oats in relatively wide rows. This method is particularly advisable when nitrogen fertilizer is applied to the oats. Seven- and 14-inch drill spacings gave the highest oat yields in most cases. The use of 21-inch drill spacings have proved impractical in most cases due to weed infestation.

When the oats are planted in rows, the legume seedlings grow more rapidly in early spring and develop a deeper root system because of reduced competition for sunlight and moisture. Consequently they are in a better position to survive low soil moisture conditions common in late summer than smaller shaded plants which have a shallower root system and cannot obtain sufficient moisture from the surface soil.

It would appear from this study that for good stands and yields of legumes, nitrogen fertilizer application to oat companion crops should not normally exceed 20 pounds per acre on broadcast oats. However, this is dependent upon the fertility level of the soil, as larger applications may be used successfully on low-producing soils. Late applications appear to be better than applications made at planting. The nitrogen should be applied with sufficient phosphorus for good legume growth. Although phosphorus does not prevent a decrease in the stand and yield of legumes from nitrogen, it does increase the yield on phosphorus-deficient soils.

Since it appears from this study that the reduction in hay yields following nitrogen fertilized oats results primarily from competition for light, any method of reducing this competition will generally result in low yields of one of the companion crops. It is necessary for the farmer to decide which of these crops is the most important to his economy. If hay and pasture crops are of primary importance, then some sacrifice in oat yield may have to be accepted to insure good stands and yields of the seeding. On the other hand if oat grain yields are of primary importance, then heavy applications of nitrogen fertilizer should be used regardless of the effect on the seeding. In this case a fall seeding might be practical. The methods investigated here for reducing this competition between companion crops offer some possibility for a compromise between these extremes.

MICROBIAL GROWTH FACTORS IN COMMERCIAL PRODUCTS OF BACTERIAL AND FUNGAL ORIGIN¹

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As research in growth factors expanded it became evident that animals, plants, and microorganisms all required the same or similar materials for normal growth and reproduction. One important result of such findings is that microorganisms can be used both as a source of vitamins and for the biological assay of vitamins. This duality of function has been realized in the recent developments leading to the isolation and large scale production of crystalline vitamin B₁₂. Certain microorganisms were used in the assay to guide the fractionation of liver from which crystalline vitamin B₁₂ was first isolated and later other organisms were used in the production of commercial quantities of B₁₂. The advantages of using microorganisms in these procedures are that the time for producing several generations is short compared to the time for producing even one generation of animals and the conditions of growth can be kept much more uniform.

The presence of growth factors in some commercial products of microbial origin was investigated. Mold bran, a fungal amylase preparation, had been found to increase yields in the alcoholic fermentation of grains when used as the saccharifying agent instead of malt. The vitamin content of mold bran was considered to explain the increase in yields that had been observed. The average amounts of the vitamins found per gram of mold bran were: biotin, 0.59 µg.; thiamin, 2.9 µg.; pantothenic acid, 12.3 µg.; inositol, 160 µg.; pyridoxine, 0.48 µg.; nicotinic acid, 113 µg.; folic acid, 1.51 µg.; riboflavin, 20 µg.

A feed supplement which was made by bacterial fermentation and which had animal protein factor (APF) activity was also investigated. There was some indication that the growth of *Lactobacillus casei* and *Lactobacillus arabinosus* was stimulated by extracts of the feed concentrate. The requirement of these organisms was not absolute and they seemed able to slowly synthesize APF. For this reason the use of these microorganisms in assay procedures for APF could not be recommended.

The growth of *Lactobacillus leichmannii* was found to depend on the presence of APF or vitamin B₁₂ in the medium. Certain substances other than those known to have APF activity interfered in the microbiological assay since they too promoted growth of the organism. Some of these interfering materials were desoxyribosides and desoxyribo-

¹ Doctoral thesis number 988, submitted August 27, 1949.

nucleic acid itself. They could not be destroyed by treatments that would leave the APF activity in the extracts since these substances were more stable than APF or B₁₂. On the contrary it was found that APF and vitamin B₁₂ activity were destroyed by autoclaving them in alkaline solution. The possibility of developing a quantitative assay for APF and vitamin B₁₂ based on this finding was suggested. The first step would consist of determining the desoxyribonucleic acid content of the extract by autoclaving with alkali and comparing the growth effect for *L. leichmannii* of the treated extracts with standards of desoxyribonucleic acid. The untreated extract would then be tested with *L. leichmannii* using standards containing the appropriate amount of desoxyribonucleic acid in addition to varying amounts of crystalline vitamin B₁₂. For use as a semi-quantitative method the first step could be omitted. This shorter procedure would be adequate for fractionation of APF in natural materials.

Lactobacillus lactis Dorner was studied for possible use in APF and B₁₂ assay methods. It was hoped that an organism might be found which would not respond to those substances which interfered with the use of *L. leichmannii*. Although early results with *L. lactis* Dorner were promising the erratic behavior of the culture made it necessary to discontinue use of this organism.

The only method that was found to release the animal protein factor activity from its conjugated form in the feed concentrate was digestion with papain and takadiastase. The activity could not be extracted by steaming in acetate buffer since both the solution obtained and the papain and takadiastase digest of the solution were inactive. Autoclaving the feed concentrate with 0.1N HCl failed to extract any material affecting the growth of *L. leichmannii*. Extraction on the steam bath with 95 per cent alcohol followed by evaporation to dryness and taking up in distilled water was also unsuccessful. The animal protein factor activity was removed from the active enzymatic digests by adsorption on norit at pH 2.0. Elution of the factor from norit could not be obtained with ammoniacal alcohol. Hot 65 per cent alcohol eluted the factor from norit.

The properties of animal protein factor and vitamin B₁₂ are almost identical and it is possible that these substances are the same or very similar. Fractionation of sufficient crude material to obtain a pure crystalline sample of APF will be necessary to decide this question.

DEFLECTION OF A FLEXIBLE PIPE CULVERT WHEN STRESSED BEYOND THE ELASTIC LIMIT ¹

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The study of the mechanics of the plastic state of matter is still in a formative stage, even though there has been a considerable amount of progress in this field in recent years. Analyses of the deflection characteristics of some of the engineering structures have been reported. However, no work is known to have been done so far on the deflection characteristics of flexible circular pipes and rings, when stressed beyond the elastic limit. The present investigation was undertaken to establish a theoretical basis for determining the deflection characteristics of a circular flexible pipe culvert when stressed beyond the elastic limit. The specific problem analyzed in this work is a circular flexible ring loaded at the top and bottom by two equal and opposite concentrated line loads. It has been considered that plastic action takes place in the top and bottom section of the loaded ring at a certain limiting load and spreads outwards as the load is increased, and that the loaded ring remains in equilibrium at any instant under the system of forces.

The two existing theories on the deformation of materials stressed beyond the elastic limit are: (1) theory of plastic flow and (2) theory of plastic deformation. In view of the relative mathematical simplicity of the theory of plastic deformation and the fact that experimental works published so far are in good agreement with both the theories, this investigation has been based on the theory of plastic deformation.

In the development of the differential equations for the loaded ring it has been assumed that the ring material obeys the idealized stress-strain law. An expression has been derived for the curvature of the loaded ring in terms of its initial radius and radial deflection. A geometrical relationship between the change in curvature of the loaded ring and its circumferential strain has been established.

The differential equation for the elastic portion of the ring has been obtained from the assumption that at any section in the elastic portion of the ring, the change in curvature is equal to the moment at the section M , divided by the flexural rigidity of the ring section, EI . The differential equation for the partially plastic portion of the ring has been obtained from the relationship between the change in curvature and the circumferential strain existing in the region.

General solutions for each of the differential equations have been

¹ Doctoral thesis number 1055, submitted May 26, 1950.

obtained containing both the complementary and the particular solutions of the equations. All the assumptions made in the development of this theory and their limitations have been discussed in the thesis. The solution of the differential equation for the elastic portion of the loaded ring has been obtained in terms of trigonometric functions, properties of the ring and its material and the applied load. The solution of the differential equation for the partially plastic portion of the ring has been obtained in terms of elliptic functions, properties of the ring and its material and the applied load.

The solutions of the two differential equations have satisfied six boundary conditions of the loaded ring from which six unknown constants, including the constants of integrations, have been calculated.

In the general solution of the differential equations, modifications have been made in the moment equations for the loaded ring due to the change in its geometry—from an initial circular shape to its deflected shape.

Comparisons have been made between the theoretical solutions for vertical and horizontal deflections and the results of tests on corrugated metal pipes. In case of horizontal deflection, the theoretical solution and the test data compared very closely over the entire range of loading— $1.45P_0$ in one case and $1.4P_0$ in the other. P_0 denotes the maximum load the ring can carry without being stressed beyond the elastic limit. In case of vertical deflection the theoretical solution and the test data compared closely—up to $1.4P_0$ in one case and $1.25P_0$ in the other.

The possibilities of the extension of this theory to flexible pipe culverts under field load conditions have been discussed. It has been suggested that with a certain amount of modification in the field load conditions, the differential equations for the flexible pipe can be obtained in forms so that their solutions may be obtainable in terms of trigonometric functions and elliptic functions.

There are two appendices included in the thesis. Appendix I is on "Deflection of a Flexible Circular Ring Due to Its Own Weight, Supported at the Base." Appendix II is on "Elastic Deflection of a Flexible Corrugated Pipe Under a Modified Field Load Condition."

In appendix I two formulas have been derived to determine the vertical and horizontal deflections of a flexible ring, supported at the base, due to its own weight. The formulas have been extended to determine the "deflection of a flexible corrugated pipe of structural steel due to its own weight, supported at the base." The extended formulas express the "deflection of a flexible corrugated pipe due to its own weight, supported at the base," in terms of its initial radius. A graph has been included to show how such deflections of a flexible corrugated pipe vary with its radius.

In appendix II, deflection equations for a flexible corrugated pipe have been established when acting under a field load condition modified from Spangler's fill-load hypothesis (1, p. 26). The modification of the loading system made possible to express the moment at any section of

the ring as a continuous function throughout the ring circumference. The equation obtained in this case for the horizontal deflection of the pipe gave closer comparison with the experimental data (1, p. 79), than Spangler's formula (1, p. 29) for determining the horizontal deflection of a flexible pipe culvert loaded according to the fill-load hypothesis (1, p. 26).

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DIFFERENCES IN THE GROWTH OF SINDHI AND CROSSES OF SINDHI WITH JERSEY, BROWN SWISS, HOLSTEIN-FRIESIAN, AND GUERNSEY CATTLE ¹

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Many attempts have been made to improve the milk yield of Indian cattle by crossbreeding with European breeds of cattle. One such project has been in operation at Allahabad Agricultural Institute, Allahabad, India. The herd at Allahabad was established in 1910. Several breeds of Indian cattle were tried, and eventually animals of the Sindhi breed were kept. Crossbreeding with European breeds was started in 1922, and four European breeds of cattle were used. In 1934 a policy of backcrossing the crossbred animals to Sindhi was initiated, and Jerseys were given preference over other European breeds in the crossing.

The growth of Sindhi and of crosses between Sindhi and Jersey, Brown Swiss, Holstein-Friesian, and Guernsey cattle, in the herd at Allahabad, has been studied. The records consisted of observations on weight and height at monthly intervals from birth to 6 months, then at 1 year and annually thereafter. A few records of birth weights were available from 1928, but most of the weights were obtained from 1934 to 1948. Records for height were available from the end of 1935 to 1948. There were more data for the weight than for the height, for early ages than for later ages, and for females than for males.

The data were grouped according to the breeds and according to the proportions of blood of European breeds in units of one-eighth. No animals of seven-eighths or purebred European breeds were available. Animals with five-eighths blood of European breeds were represented by those with Jersey breeding only. The crosses with Guernsey breeding were represented in one-eighth, two-eighths, and four-eighths groups only. Analyses of variance and covariance were made. Because the data were disproportional in various classifications and because some classes were missing altogether, the least squares estimates were obtained by the method of fitting constants.

The height and weight of male calves were studied for observations at birth only. The Sindhi male calves averaged 25.21 ± 0.14 inches in height at birth (66 observations), and their mean birth weight was 44.5 ± 0.9 pounds (72 observations). The height of crossbred male calves at birth was 24.99 ± 0.13 inches (173 observations), and their birth weight was 46.0 ± 0.8 pounds (202 observations). The differences

¹ Doctoral Thesis number 1008, submitted December 5, 1949.

between Sindi and the crossbred animals in the height and the weight were not significant. Within the crossbred male animals, the differences attributable to the four breeds of European cattle and to the proportion of European blood were significant. The differences due to interactions between these two variables were not significant.

The average height of female Sindhi calves was 24.85 ± 0.15 inches (103 observations) at birth and 43.94 ± 0.4 inches (18 observations) at 3 years. The height of female crossbred calves was 24.94 ± 0.10 inches (298 observations) at birth and 44.41 ± 0.2 inches (67 observations) at 3 years. The differences between the two groups were not significant at any age. Analyses of variance for height at birth, at 6, 12, 24, and 36 months were carried out. The differences attributable to the proportions of blood of European breeds were significant at birth only, while the differences due to the breeds of European cattle were significant at birth and at 3 years. The significant differences at 3 years were indicative of the difference in ages at which the crosses involving the various breeds tend to reach skeletal maturity.

The average weight of Sindhi female calves was 42.5 ± 0.8 pounds at birth (115 observations) and 447 ± 13 pounds at 2 years (58 observations). The mean weights of crossbred females were 44.7 ± 0.6 pounds (365 observations) and 500 ± 7 pounds (194 observations), respectively, at birth and at 2 years.

Analyses of variance for weights at each age observed were carried out for 233 females who had continuous records from birth to 24 months. The mean weights of Sindhi and crossbred female calves differed significantly at all ages studied except at 1 and 4 months. The differences in the weight of the crossbred female calves attributable to the varying proportions of blood of European breeds were not significant at 2 months. They were significant at other ages. The differences due to breeds of European cattle were significant at all ages studied except at 24 months. The differences due to interactions between these two variables were not significant at any age.

Analyses of covariance between weights of the 233 female calves at different ages were carried out. The errors of estimate from regressions of weights at later ages on weights at earlier ages showed that the mean squares for differences between Sindhis and crosses were generally significant. In the data for crosses, the differences between varying proportions of European cattle in the later weights were generally significant even after adjustment for regression on the earlier weight. This indicates that knowledge of differences due to proportion at earlier ages was not wholly adequate for predicting these differences at later ages. The breed differences up to 3 months did not persist, but the breed differences which appeared at about 3 months or soon afterward did persist into the later ages. The interactions between breeds and proportions were not significant generally.

The lack of such significant interactions indicates that the genetic factors affecting growth in height and weight were additive in nature.

Crossbred calves grew more rapidly in weight than Sindhi calves at all ages studied except between birth and 1 month and between 6 and 12 months. Since these periods were characterized by changes in environments, it appears that the crosses were slower than the Sindhi animals in adapting themselves to these changes.

Guernsey crosses did not grow so well as others in height and weight. Holstein-Friesian crosses grew to be the largest animals. Of the four types of crosses studied, the growth of Jersey crosses was most similar to that of Sindhi animals. The animals with 50 per cent European blood had the fastest growth, and animals with 75 per cent blood of European breeds were generally unthrifty.

There was some evidence that the influence of maternal environment was strong at birth but dwindled as the animals grew older. Heterosis of the dam was accompanied by heavy weights of calves at birth, but the heterosis of calf was associated with greater gains at later ages. There were indications that increase in fraction of Sindhi blood from three-eighths to six-eighths at the expense of European blood resulted in greater gains in weight.

EXTRACTION OF CERTAIN OIL-BEARING MATERIALS WITH TRICHLOROETHYLENE¹

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Research at Iowa State College during the past several years has resulted in the development of a successful method of removing oil from soybeans by use of trichloroethylene. The success of this process with soybeans led to interest in extracting oil from other oil-bearing materials.

The present work involved laboratory and pilot plant extractions of seven different materials. The pilot plant extractor consisted of a Redler conveyer chain moving flaked material through a rectangular tube countercurrent to the solvent. The extracted flakes passed through a drainage section, through two driers and out of the system. Miscella was removed near the flake inlet, concentrated in a rising film evaporator tube and then stripped of the remaining solvent with steam in a packed column. Rate of extraction data were determined in the laboratory both in Soxhlet extractors and in a tube designed for cold solvent extraction. The data were plotted as per cent residual oil versus time of extraction and were compared with pilot plant data.

In pilot plant extractions, wheat germ flakes 0.005 inches in thickness were reduced from 13.9 per cent oil to 0.76 per cent in 15.3 minutes, and to 0.61 per cent in 25.5 minutes. The pilot plant capacities were 84 and 150 pounds per hour respectively for the above extractions. Use of vacuum equipment for removal of solvent was recommended in order to prevent damage to the tocopherols in the oil. Laboratory rate of extraction data indicated a very rapid removal of oil by hot solvent.

Milkweed seed flakes had a very slow rate of extraction. Extraction of flakes 0.006 inches in thickness in the pilot plant reduced the oil content from 25.6 per cent to 4.6 per cent in a 15.3-minute extraction and to 3.5 per cent in 25.5 minutes. Capacities for the above extraction times were 41 and 59 pounds per hour, respectively. The slow diffusion rate for milkweed seed precluded the possibility of reducing the residual oil content to 1 per cent in less than one hour. The filtering of milkweed seed oil-trichloroethylene miscella required the use of filter aid.

Sorghum grain contains both an oil and a wax. Extraction of flaked sorghum germs was successful with the oil content being reduced from 10.3 to 1.5 per cent in 15.3 minutes. A good quality of oil was obtained. The best flakes were obtained from material containing about 11 per cent moisture. The extraction of the wax from sorghum bran was unsuccessful due to fines in the bran which prevented proper flow of solvent.

¹ Doctoral thesis number 1063, submitted June 2, 1950.

Flaked peanuts were found unsuitable for extraction in the pilot plant due to a tendency of the flakes to disintegrate shortly after contacting the solvent. Peanut oil-trichloroethylene miscella was difficult to filter.

Several types of waste waxed paper were extracted in the laboratory and in the pilot plant. The residual wax contents for pilot plant runs ranged from 1.1 per cent for bread wrap to 2.5 per cent for laminated carton material. Pilot plant capacity ranged from 10 to 40 pounds per hour. The driers of the pilot plant became plugged frequently when strips of paper were present in the material being extracted. Paper which had been cut into pieces and screened for size proved to be the most satisfactory. Insulation of all pipes carrying concentrated wax-solvent miscellas was recommended to prevent cooling and solidification within the pipes.

Safflower seed was investigated and found unsuitable for forming flakes due to the high oil content of the kernel. Extraction of seed which had been prepressed in an expeller gave promising results. Ground expeller cake with an oil content of 10 per cent was reduced to 1.6 per cent in 15.3 minutes in the pilot plant. A capacity of 248 pounds of ground cake per hour was obtained.

Extraction of cottonseed oil was accomplished both from the whole seed and from the meats. In each case, about the same percentage of the total oil was extracted. Whole seed, when flaked, was extracted at the rate of 69 pounds per hour in the pilot plant. The residual oil content of flakes 0.012 inches in thickness was 1.1 per cent after a 25.5-minute extraction time. Flaked meats were found to give a higher pilot plant capacity, from 92 to 96 pounds per hour being extracted to a 2.6 per cent residual oil in 25.5 minutes. An extraction time of 61 minutes left 1.7 per cent oil in the meal. Drying of the flakes before extraction resulted in better retention of flake structure during handling and extraction. Rate of extraction data determined in the laboratory indicated that with hot solvent the residual oil content could be reduced to 1 per cent in about 50 minutes.

A very dark crude oil was obtained from all cottonseed oil extractions with trichloroethylene. In color removal studies, it was found that dilute sodium carbonate solutions partially decolorized cottonseed oil-trichloroethylene miscella but solutions 5 per cent or stronger intensified the red color.

Oil which was concentrated and stripped of solvent at atmospheric pressure failed to refine to an acceptable color. The use of vacuum equipment for desolventization of the miscella yielded a crude oil which, although dark colored, refined to a light-colored prime oil. The oil also could be bleached by standard methods. The refining of trichloroethylene-extracted cottonseed oil gave a firm soap stock with a minimum of occluded oil, thus greatly facilitating the refining procedure.

This work has demonstrated that the extraction process, developed at Iowa State College using nonflammable trichloroethylene, can be adapted to the extraction of a number of different oil-bearing materials.

FACTORS CONCERNED IN TYROSINE METABOLISM¹

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Early studies of the intermediary metabolism of tyrosine were mainly concerned with the investigation of alcaptonuria. This disease was characterized by the incomplete utilization of the amino acid and the consequent appearance of homogentisic acid in the urine of the alcaptonuric patient. With the finding that the vitamin C-deficient guinea pig also failed to metabolize tyrosine completely, a new approach to the problem under controlled experimental conditions was made possible.

The incomplete metabolism of tyrosine by the vitamin C-deficient guinea pig results in the appearance of homogentisic, *p*-hydroxyphenylpyruvic and *p*-hydroxyphenyllactic acids in the urine. It has been reported that, in addition to vitamin C, folic acid will prevent the appearance of these metabolites. Injection of liver extracts used in the treatment of pernicious anemia has also been reported to decrease the excretion of tyrosine metabolites by the vitamin C-deficient guinea pig.

The present study is an attempt to investigate further the mechanism by which these three factors act on tyrosine metabolism in the vitamin C-deficient guinea pig. Liver extracts containing no vitamin C or folic acid were studied extensively in order to analyze the testing procedure, study the relationship between their antipernicious anemia unitage and their activity in the tyrosine metabolic system, investigate the nature of the substance in the extracts responsible for their action, and finally, examine their action on blood constituents under various experimental conditions.

As the first step of a comprehensive program designed to study the mode of action of these three factors using the isotopic amino acid labelled in various positions, L-tyrosine containing N¹⁵ was fed to control, folic acid-supplemented, and vitamin C-supplemented animals. The nitrogenous constituents of the urine of these animals were then studied to determine whether the decreases in metabolite excretion observed with vitamin supplementation also included changes in the content or distribution of the tyrosine nitrogen in the urine. Eventually, it is hoped that work along these lines will result in the assignment of specific chemical functions in the tyrosine metabolic scheme to these factors.

In these experiments, male guinea pigs were housed in individual

¹ Doctoral thesis number 1025 submitted January 17, 1950.

wire mesh cages supported in large funnels. Feces and food particles were caught in fine mesh screening underneath the cage, while the urine ran into a collecting bottle containing 10 ml. of 2.0 *N* hydrochloric acid. After two to three days on a basal diet containing insignificant amounts of vitamin C, the animals were fed daily supplements of 200 mg. per 100 g. of body weight of L-tyrosine. At the end of each 24-hour period, the urine samples were analyzed for keto acid by a modification of the method of Penrose and Quastel and for total tyrosyl (phenol) value by a modification of the method of Folin and Ciocalteu.

The experimental procedure followed was to feed the tyrosine supplement and analyze at the end of each 24-hour period until the keto acid excretion was 30 per cent or more of the administered tyrosine. The various vitamin supplements were then given at the start of the next 24-hour period and the analyses continued. In the isotope experiments, a similar procedure was followed except that isotopic tyrosine was fed instead of the normal amino acid and the animal was killed after 24 or 48 hours. In these isotope experiments, in addition to analyses of the urine for keto acid and tyrosyl value, the total nitrogen, ammonia nitrogen, urea nitrogen, and residual nitrogen were determined and also analyzed for their content of isotopic nitrogen. The isotopic nitrogen content of the feces, uneaten food, and intestinal contents were also analyzed.

The results of this study confirmed the finding that liver extracts, used in the treatment of pernicious anemia, decreased the excretion of tyrosine metabolites by the vitamin C-deficient guinea pig. Suitable extracts reduced the metabolite excretion by approximately half. A vitamin B₁₂-charcoal adsorbate in a soybean flour carrier, when fed orally, had a similar action on metabolite excretion. The effect produced was approximately proportional to the amount of vitamin B₁₂ administered. It has been tentatively concluded that the activity of liver extracts is due, at least in part, to their vitamin B₁₂ content.

The decreases in metabolite excretion caused by liver extract were observed primarily in the 24-hour period following injections and could not be maintained by continued injections. The decreases observed were not always exactly proportional to the antipernicious anemia unitage administered. It has been concluded that other factors probably operate in conjunction with the activity of the liver extracts to produce the metabolite decreases.

The action of vitamin C or cobalt salts in conjunction with these liver extracts was studied. Daily administration of 1 mg. of vitamin C resulted in larger decreases in metabolite excretion after liver extract injection. The cobalt salts used had no effect on the action of the liver extract. Injection of a liver dialyzate resulted in irregular decreases in metabolite excretion.

A purified diet was prepared and the action of these factors on metabolite excretion studied with this basal diet. Vitamin C and folic acid supplementation resulted in the usual cessation of metabolite

excretion. In the one experiment with liver extract injections, no change in excretory values occurred.

Studies of blood constituents were made to determine whether the action of liver extracts on tyrosine metabolism was correlated with changes in red blood cell counts, total nitrogen and hemoglobin. A suggestion that decreases in metabolite excretion were correlated with increases in the number of red blood cells was noted.

Further studies of the action of vitamin C and folic acid on tyrosine metabolism were made using isotope-labelled amino acid. L-Tyrosine containing 6.01 atoms per cent excess N^{15} was fed to control and vitamin-supplemented animals. It was found that 80 to 90 per cent of the isotopic tyrosine nitrogen was absorbed from the digestive tract in the first 24 hours.

Approximately 30 per cent of the isotopic tyrosine nitrogen absorbed by unsupplemented, vitamin C-supplemented, and folic acid-supplemented animals appeared in the urine in the first 24 hours. An additional 5 per cent was excreted in the second 24-hour period. It was concluded that the decreases in metabolite excretion produced by vitamin C and folic acid were not accompanied by changes in the amount of isotopic tyrosine nitrogen excreted.

However, the distribution of isotopic nitrogen in the urinary fractions studied differed when the supplement was varied. The nitrogen derived from the urea fraction contained more isotopic tyrosine nitrogen in the case of vitamin-supplemented animals than controls. The urine fraction which contained the greatest concentration of isotopic tyrosine nitrogen was ammonia in the case of vitamin C-supplemented animals, but this was not true for control or folic acid-supplemented animals.

EFFECTS OF SELECTED DIETS AND METAMORPHOSIS ON LIPID COMPOSITION OF *DERMESTES MACULATUS* DEG.¹

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The lipid content of insect diets varies to a great extent. Some insects are able to complete their development on a medium containing only traces of fat, and synthesize body lipids from diet proteins and carbohydrates. A few species have been found to require as a growth factor a substance present in the unsaponified fraction of lipids. In most cases, this substance has been identified as a sterol.

The nature of stored fat in insects seems to be influenced by the following factors: development, nutrition, environmental temperature, sex, systematic position, starvation, hibernation, cold hardiness, and migration. A review of the available literature on the lipid content of insects disclosed that the major components of the fatty acid fraction appear to be oleic, linoleic, palmitic, and stearic acids. Less saturated acids, and acids of both lower and higher molecular weight, have been identified in the extract of insects or insect products. Generalizations as to the phospholipid and sterol fractions were not warranted by the review.

After extraction of insect samples with diethyl ether-ethyl alcohol (1:3) in an apparatus especially designed for this lipid study, the various lipid components were separated by a modification of the methods of previous workers. The components were determined by oxidation with potassium dichromate and were calculated by the method commonly used in blood lipid determinations. The moisture content of replicate samples, and the degree of unsaturation of non-phospholipid fatty acids were also determined. The above procedures of lipid analysis were tested with purified compounds and applied to a study of the effects of metamorphosis, ageing, and selected diets on the lipid composition of *Dermestes maculatus*.

Determination of the lipid composition of various stages of *D. maculatus*, when the medium was sardine fish meal, indicated a few trends. Phospholipid, total fatty acids, neutral fat, and total lipid decreased as a percentage of the fresh weight during the period of development from egg to 10-day larva. Total cholesterol increased during the same period. After an initial increase in the 20-day larva, phospholipid content decreased until shortly after pupation. An increase during the pupal stage was followed by a decrease in adult life. Total cholesterol remained fairly constant during larval development after a decrease from the 10-day to the 20-day larva. A slight increase during pupation

¹ Doctoral thesis number 1012 submitted December 12, 1949.

continued in adult life. Total fatty acids, neutral fat, and total lipid increased as a percentage of live weight during larval life and reached a maximum shortly after pupation. A diminution occurred in the latter part of the pupal stage and in adult life. The iodine number of non-phospholipid fatty acids increased from the egg stage to the 10-day larva; decreased during the remaining portion of larval development; increased during pupal life; and decreased again during adult life. The water content decreased steadily during larval development and increased during pupation and adult life.

When calculated as a percentage of total lipid, phospholipid increased during early development, decreased in late larval development and the first part of the pupal stage; increased during the remaining part of the pupal stage and adult life of the male; and increased during female life after an initial decrease. With a few variations, total cholesterol and cholesterol ester fatty acids declined in relation to total lipid in larval life and increased in pupal and adult life. Total fatty acids and neutral fat increased as a fraction of total lipid during the larval stage and decreased during the pupal and adult stages.

Analysis of prepupae reared on various media indicated a few effects of diet on body lipids. Larvae matured on ether-chloroform extracted fish meal contained a lower percentage of neutral fat, total fatty acids, and total lipid than those reared on unaltered fish meal. The phospholipid and total cholesterol contents exhibited very little change. Addition of 1.0 per cent cholesterol to extracted fish meal resulted in an increase in the content of neutral fat, total fatty acids, and total lipid. Addition of 10 per cent glycerol, stearic acid, or linoleic acid to the diet of extracted fish meal plus cholesterol caused a diminution in phospholipid, total fatty acid, neutral fat, and total lipid contents. Addition of oleic acid to the same basic medium produced decreases in phospholipid, total fatty acid, and total lipid contents. Total cholesterol increased markedly. When the medium contained 25 per cent stearic acid, the prepupae contained a higher percentage of neutral fat, total fatty acids, and total lipid as compared to prepupae on a medium containing 10 per cent stearic acid. The iodine number of non-phospholipid fatty acids increased with the addition of more unsaturated acids. A diet consisting of fructose, yeast, and cholesterol produced prepupae with a higher percentage of phospholipid, total fatty acids, neutral fat, and total lipid than a diet of sardine fish meal.

Calculation of lipid components as a percentage of total lipid indicated a few trends. Neutral fat and total fatty acids accounted for a smaller percentage of total lipid in larvae reared on extracted fish meal than in larvae reared on unaltered fish meal. Phospholipid and total cholesterol increased. Addition of 1.0 per cent cholesterol to extracted fish meal resulted in a decrease in the phospholipid fraction and an increase in the neutral fat fraction. Addition of glycerol, corn oil, stearic acid, oleic acid, or linoleic acid caused a decrease in the phospholipid fraction when calculated as a percentage of total lipid. Total cholesterol exhibited an increase.

AN INVESTIGATION OF THE FEMALE GENITALIA AS
TAXONOMIC CHARACTERS IN THE
MIRIDAE (HEMIPTERA) ¹

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The female genitalia of the species of the family *Miridae* have never been investigated taxonomically. Two structures of the female genitalia have been studied. These are the sclerotized rings lying on the dorsal wall of the bursa copulatrix, and the posterior wall of the bursa copulatrix.

One hundred and eight species representing sixty-seven genera and nine subfamilies have been studied. The genital parts investigated reveal characters of taxonomic and phylogenetic value to be present. The *Bryocorinae* are considered to represent the most primitive condition. The *Phylinae* and *Dicyphinae* are considered generalized types from which the more specialized *Capsinae* and *Orthotylinae* have arisen.

Taxonomic characters of value in defining genera and species, as well as higher groups, are present in the female genitalia.

Special attention has been given to the subfamily *Capsinae* where fifty-eight species representing thirty-three genera have been studied. Five groups have been ascertainable within the subfamily. These groups have been separated primarily upon characters found in the posterior wall of the bursa copulatrix.

¹ Doctoral thesis number 1034, submitted March 10, 1950.

INFLUENCE OF INBREEDING ON EGG PRODUCTION IN THE DOMESTIC FOWL¹

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Inbreeding among animals in general results in a decline in vigor. In the domestic fowl, inbreeding has its most adverse influence on egg production, hatchability, and vitality. The primary purpose of this study was to estimate the regression of egg production on inbreeding and to determine whether different lines of breeding are characterized by different regressions.

The data for this study were collected from 1932 through 1945 on White Leghorns bred at the Iowa State College Poultry Farm. The birds were classified into twenty-three inbred lines comprising 6,775 individuals and three non-inbred lines comprising 3,224 individuals. Only nine introductions of outside stock took place over the 15 years covered in these data. The twenty-three inbred lines studied showed varying amounts of relationship to each other. This relationship would make the line differences smaller than would be expected with stock from independent sources.

The effect of inbreeding on pause periods of 8 days or more and pauses of less than 8 days was determined on the 1939 data. The mean annual production of 51 inbreds and 63 single crosses was 76 eggs and 144 eggs, respectively. The records on each of these birds extended over 365 days following their first egg. The inbreds and single crosses were significantly different in total days spent in pauses of 8 days or more. However, these two groups of birds did not differ in their net rate of production. Net rate was based on the days after first egg, exclusive of pauses of 8 days or more.

The pullet year egg production was divided into three periods according to season. The highest rate of egg production occurred in the fall. The regression of egg production rate on inbreeding was approximately the same for each seasonal period.

The regression of egg production rate on inbreeding was estimated first for the birds in the inbred lines without corrections for lines, years, or line-year interactions. This regression and its standard error was $-.30 \pm .10$, which indicates that for each increase of 1 per cent in the coefficient of inbreeding, rate of egg production falls off by .3 per cent. Regression was also estimated from mean production rates of birds classified according to inbreeding coefficients. Each category contained birds within a 5 per cent range of inbreeding coefficients. The regression

¹ Doctoral thesis number 976, submitted July 1, 1949.

of the mean egg production rate on these inbreeding categories was $-.28 \pm .03$.

The relationship between inbreeding and egg production rate was also estimated by the regression of the change in mean egg production rate between offspring and parent's family on the change in inbreeding between offspring and parent. This regression was $-.35 \pm .01$ and is independent of line differences after the first generation of a new line.

The most reliable estimate of the regression of egg production rate on inbreeding coefficient was obtained by the method of least squares. The model used was:

$$\text{Egg Production Rate} = \text{population mean} + \text{line effect} + \text{yearly effect} + \text{line-year interaction} + \text{inbreeding regression} + \text{error}.$$

The general regression of egg production rate on inbreeding was estimated to be $-.43 \pm .04$ by this method which is the most reliable estimate of the average regression, since it is the least influenced by environmental factors. Corrections were made for line, year, and line-year interaction in this regression. These results indicate that egg production rate is expected to decrease 4 per cent for each 10 per cent increase in inbreeding coefficients.

The least squares analysis showed that specific lines have regressions which are significantly different from a general regression. However, the general regression is a good measure of the average effect of inbreeding on egg production for most lines.

Interaction between lines and years was found to be highly significant by the least squares analysis. This means that all inbred lines do not respond in the same manner to a given environment. Because of this specific reaction to yearly effect, the ranking of inbred lines according to egg production varied from year to year.

An analysis of year-line subclass means showed the variance components of lines to be approximately 10 times as great as that of years on line-year interaction.

Inbreeding is a much stronger force in decreasing egg production than selection is in increasing production. Assuming 30 per cent of the breeding flock are needed for replacements, only 2 to 5 per cent inbreeding per generation can be counteracted by selection. In a closed flock consisting of only five or six males, the inbreeding effect would probably cancel all selection pressure to increase egg production.

PRODUCTION OF SODIUM HYDROXIDE SOLUTION BY ION EXCHANGE ¹

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An investigation was carried out to determine materials and procedures by which the phenomenon of ion exchange could be utilized in a process to produce pure dilute sodium hydroxide solution from sodium chloride. The solutions thus produced are intended for direct plant use with the consequent saving of evaporation and freight costs.

The process, as developed in this investigation involves the use of strontium hydroxide as an intermediate. Exchange of strontium ions from hot strontium hydroxide solution for sodium ions on a cation exchange resin, followed by precipitation of the excess strontium hydroxide by cooling, produces a pure dilute sodium hydroxide solution of about 3.5 per cent. The resin is then stripped of its strontium ions by passing sodium chloride solution through it. The effluent solution from this regeneration step contains the strontium ions which may be precipitated as basic strontium chloride. Reconversion of this chloride to the hydroxide is accomplished by calcining in the presence of steam during which hydrogen chloride is given off and condensed with the steam to give a 10 per cent solution of hydrochloric acid. The over-all process is thus an indirect means of bringing about the hydrolysis of sodium chloride into sodium hydroxide and hydrochloric acid at a temperature that is industrially available and considerably lower than that required for the direct hydrolysis of sodium chloride.

Experimental exploration of this ion-exchange process was conducted with the aim of finding the best exchangeable hydroxide, the most suitable ion exchange material, and the optimum conditions for exchange and recovery of the exchangeable hydroxide.

Preliminary examination of the solubilities and costs of the various soluble hydroxides indicated that only the hydroxides of calcium, strontium, barium, and ammonium could be suitable for use as the exchangeable hydroxide. Experimental determination of the exchange characteristics of these four hydroxides revealed that only strontium hydroxide was capable of producing an appreciable concentration of sodium hydroxide by ion exchange. Calcium hydroxide produced a maximum sodium hydroxide solution concentration of only 0.6 per cent. This was obtained by stirring a thick suspension of calcium hydroxide powder with the sodium loaded resin, and filtering. Attempts were made to produce a higher concentration of sodium hydroxide by utilizing mul-

¹ Doctoral thesis number 1059, submitted June 1, 1950.

tiple contact of the resin with calcium hydroxide solution or suspension and varying the temperature, contact time, and solvent. None of these attempts was successful. The use of calcium hydroxide in an ion exchange also yielded unsatisfactory results. The weak exchange power of calcium hydroxide was shown to be due to its low solubility. With ammonium hydroxide almost no cation exchange at all took place. This is believed to be due to the very low dissociation of ammonium hydroxide in water. The exchange characteristics of dilute barium hydroxide at room temperature were similar to those of strontium hydroxide under similar conditions. However, a hot concentrated solution of the hydroxide was found to be necessary to produce an appreciable concentration of sodium hydroxide. Under these conditions the behavior of barium hydroxide was decidedly inferior to that of strontium hydroxide. Barium hydroxide has a strong tendency to be adsorbed as the entire molecule on the resin from concentrated solution, concurrently with exchange of the cations. Furthermore, upon precipitation of the excess unexchanged barium hydroxide by cooling, much of the sodium hydroxide that formed in solution by ion exchange was also precipitated in the form of double base $\text{Ba}(\text{OH})_2 \cdot \text{NaOH}$. As a result, the final maximum sodium hydroxide concentration was only 0.7 per cent NaOH. Strontium hydroxide exhibited none of these undesirable characteristics and produced pure sodium hydroxide solutions with concentrations as high as 3.5 per cent. Strontium hydroxide was therefore selected as the best exchangeable hydroxide.

A peculiarity of cation exchange from hydroxide solutions is the tendency of hydroxyl ion to be adsorbed on the resin along with the cation during exchange. In the case of barium hydroxide this is largely due to the physical adsorption of the entire molecule on the resin. With strontium hydroxide part of the hydroxyl ion, adsorption is due to neutralization of weakly acidic hydrogen atoms in the resin, but this adsorption also occurs on resins of the polystyrene type which do not contain weakly acidic hydrogen atoms. Hydroxyl ion adsorption in the latter case is due to the formation of the $(\text{SrOH})^+$ ion which enters into cation exchange along with the Sr^{++} ion. When the resin is subsequently regenerated with sodium chloride solution, these ions are desorbed from the resin and the hydroxyl ion reappears in the regenerant solution. The presence of this hydroxyl ion in the regenerant solution is beneficial since it aids in the precipitation of strontium as basic strontium chloride.

Of those cation exchange materials that are capable of withstanding hot alkaline solutions, three representative resins were selected for test. These were Dow MX, a sulfonated phenol-formaldehyde resin; Amberlite IRC-50, a carboxylated polystyrene resin; and Dow HCR, a sulfonated polystyrene resin. The Dow HCR resin proved to be the most desirable for the purpose at hand. With it, a 3.5 per cent sodium hydroxide solution was produced as compared with 2.1 per cent with Dow MX and 1.0 per cent with Amberlite IRC-50. The Dow HCR resin also

showed a higher exchange capacity and a lower hydroxyl ion adsorption than the other two resins. It therefore was selected as the best exchange material available at present.

Using strontium hydroxide and Dow HCR resin, rate and equilibrium studies were made in a specially designed ion exchange unit to determine optimum conditions for exchange. It was found that exchange reaches practical equilibrium within a few minutes and that temperature has very little effect on the rate or equilibrium of exchange. For optimum results the ratio of initial milli-equivalents of strontium in solution to grams of sodium-loaded resin should be 2.8, and the initial concentration of strontium in solution should be 1.3 equivalents per liter of solution. The volume of solution should be kept to a minimum.

The strontium chloride in the effluent solution may be separated from the sodium chloride by a crystallization process involving evaporation, or by precipitation as basic strontium chloride. The latter method is preferred because it does not involve evaporation.

The separated strontium chloride may be reconverted to strontium hydroxide by calcining at 1700°F. in the presence of steam and finely divided silica in the course of which hydrogen chloride is given off.

A quantitative flow sheet for the process, based on the experimental data, was devised. A rough evaluation indicated that in order for the process to be economically attractive, the plant in which it is located must be able to consume both the sodium hydroxide and the hydrochloric acid produced.

EFFECT OF VITAMIN B₁₂ AND OF ANIMAL PROTEINS ON THE HISTOPATHOLOGY AND HEMATOLOGY OF CHICKS RECEIVING AN ALL-PLANT RATION¹

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Growth response has been the principal criterion in evaluating the completeness of the various nutrients in rations including vitamin B₁₂. This experiment was designed to use other criteria in addition to growth response in determining adequate levels of required nutrients to produce maximum growth.

Three groups of chicks (10 in each group) received the basal ration whose protein was of all-plant origin, three other groups received the same ration except 3 per cent liver meal replaced 3 per cent soybean meal, and the remaining three groups received 0.5 per cent APF concentrate in place of 0.5 per cent soybean meal. The vitamin B₁₂ activity of the APF concentrate was 12.5 mg. per pound as measured by the *Lactobacillus lactis* Dorner (L.L.D.) method of assay. Thus, the latter ration contained 62.5 µg. of vitamin B₁₂ per pound.

Erythrocyte, leucocyte, and differential counts were determined on blood samples from the ninety chicks. Hemoglobin determinations, hematocrit readings, and sedimentation rates of erythrocytes at ½, 1, 2, 3, and 6 hours were also made. These observations were made again on blood samples from four chicks in each pen toward the end of the experiment in order to determine age effect. Statistical analyses of these data were made. Sciatic nerves from ten chicks (two from each pen) were examined between crossed prisms of a polarizing microscope at the point of greatest birefringence. Various tissues from these eighteen chicks were stained with hematoxylin and triosin. Corresponding tissues were also stained according to the Marchi method.

The treatments produced a highly significant difference statistically in the weight gains of the forty-five female chicks for the first 10 weeks of the experiment and a significant difference at 12 weeks of age. The weight gains of the forty-five male chicks were found to be significantly different among the chicks receiving the three rations for the first 2 weeks and no significant difference during the balance of the experiment. A highly significant difference was present between weight gains of female chicks fed the basal and liver meal rations throughout the experiment, whereas this difference existed only at 2 weeks of age in male chicks. A significant difference was present between the weight

¹ Doctoral thesis number 1036, submitted March 13, 1950.

gains of male chicks fed the basal and liver meal rations at 4 and 8 weeks of age. A highly significant difference existed between the weight gains of female chicks fed the basal and APF rations at 4 and 6 weeks of age while a significant difference occurred between the weight gains of female chicks at 2, 8, and 10 weeks of age and at 2 weeks of age for male chicks. Female chicks did not gain as rapidly as male chicks and the standard errors of the means were smaller for female chicks.

Environmental effects produced a highly significant difference in the weight gains of male chicks at 4, 6, and 8 weeks of age and a significant difference at 10 weeks. This experiment indicates that female chicks should be better assay animals for vitamin B₁₂ than male chicks due to the smaller standard error of the means of weight gains and the absence of significant environmental effects.

The feed efficiency of the chicks fed the liver meal ration was the best throughout the experiment followed in order by those fed the APF and basal rations to 8 weeks of age, whereas the chicks fed the APF ration showed the poorest feed efficiency thereafter. A highly significant difference was present between the feed efficiency of chicks fed the basal and liver meal rations and also between those fed the APF and liver meal rations while a significant difference in feed efficiency did not exist between the chicks fed the basal and APF rations.

A significant difference was found between the hemoglobin levels of the female chicks fed the basal and liver meal rations while no significant difference existed between the hemoglobin levels of chicks fed the basal and APF rations and also between those fed the liver meal and APF rations. The hemoglobin levels of the male chicks fed the three rations were not significantly different. The packed erythrocyte volume of female chicks fed the basal and liver meal rations approached the 5 per cent level of significance. Treatment effects on erythrocyte counts, total leucocyte counts, differential counts, sedimentation rates, plasma volume, and packed leucocytes and thrombocytes were not significant. Environmental effects produced a highly significant difference in the basophil counts of male chicks, a significant difference in the sedimentation rate of erythrocytes of male chicks in one-half hour, and in female chicks a significant difference in the total leucocyte counts, lymphocyte counts, and sedimentation rate of erythrocytes in 2 hours. Age (21 to 37 days) produced a highly significant difference in the hemoglobin levels of chicks and a significant difference in the eosinophil counts, whereas the remaining blood determinations had not changed significantly.

Eight gizzards from the eighteen chicks autopsied presented erosions in the keratinized portion of the epithelium while the glands in the tunica propria of the mucous membrane appeared normal microscopically. Grossly, a majority of the hearts showed areas of grayish discoloration in the muscle under the epicardium which on microscopic examination appeared to be an areolar-type of connective tissue joining the epicardium to the normal muscle fibers. This condition was attributed

to the needle puncturing the heart in obtaining blood samples. The ceca of the chicks were unusually long, varying from 7 to 11 inches in length but showing no pathologic changes microscopically.

The leg weakness of chicks fed the basal and APF rations was not associated with myelin sheath degeneration. Treatments produced no appreciable change in the degree of myelin sheath degeneration in sciatic nerves of chicks fed the three rations as observed between crossed prisms of a polarizing microscope and by the Marchi method of staining. The Marchi method of staining and the hematoxylin-triosin stain revealed a fatty change in the liver, the greatest change appearing in the livers of chicks fed the APF ration followed in order by those fed the liver meal and basal rations. The hearts from two chicks fed the APF ration and one chick fed the liver meal ration showed a fatty change by the Marchi method while a majority of the other chicks fed the three rations showed only a trace of fatty change in the heart muscle fibers which was considered to be of no pathologic importance.

The Marchi method of staining revealed the presence of fat in ova, interstitial cells of testis, and in the enzyme-secreting cells of the pancreas and proventriculus which was considered to be normal in the first two locations and either normal or of minor importance in the latter two. Pyknotic nuclei and albuminous degeneration were observed in the tubule cells of the kidneys stained with hematoxylin and triosin. The kidney and liver changes and the occurrence of six cases of perosis in male chicks suggest that the rations may have been deficient in choline. The increased cellularity in the center of the capillary tufts in the glomeruli of chicken kidney, as compared with mammalian kidney, is believed to be a normal characteristic. Hyalinized areas appearing in the medulla of the thymus near Hassall's corpuscles are believed to be normal for the chick and these areas may eventually become Hassall's corpuscles.

SHEAR LAG IN TENSION PANELS AND BOX BEAMS ¹

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In aeronautical engineering, the solution of the shear-lag problem became necessary with the development and general adoption of the all-metal airplane. The term, shear lag, came into use because shearing stresses in the cover sheet or "skin" of the airplane develop to a lesser extent than is predicted by application of the conventional engineering shear-stress formula. The skin shear stresses were therefore said to lag, and it has become common practice in this country to refer briefly to such behavior as shear lag.

In addition to the shear distribution, another feature of paramount interest in shear-lag behavior is the resulting nonuniformity in distribution of normal stress. As a result of this nonuniform stress distribution, the actual strength of a stressed-skin structure is materially less than the apparent strength computed from the conventional flexure formula.

Because of the real need for maximum efficiency in military and commercial airplanes, numerous investigations have been carried out in several nations in order to devise solutions of the shear-lag problem. A large number of these studies have been government sponsored and financed. Such investigations were under way in Germany prior to 1930 and were continued until the early part of 1945. Engineers in this country have been studying the problem since 1930 with the bulk of the investigation being conducted by scientists of the National Advisory Committee for Aeronautics. Most of the research in England has been done since 1937 under the auspices of the British Aeronautical Research Committee. In spite of the many investigations, existing solutions are approximate; and experimental studies have revealed that their use may frequently lead to large errors in the analysis of airplane structures.

In the present investigation, solutions are obtained which are in excellent agreement with the experimental results. The structures analyzed are symmetrical, double-flanged, tension panels and two-spar, flat-cover, box beams with no stringers. These analyses can be extended without difficulty to more complicated structural arrangements, which is to be done in future papers. For box beams, general solutions are presented for the conditions that obtain before and after buckling of compression cover sheets. In addition, for use in the general solutions, expressions are developed for five specific loading conditions: (1) a concentrated load P at the beam tip, (2) a uniform load of intensity w distributed over the full span, (3) a concentrated load P at any in-

¹ Doctoral dissertation number 1023, submitted Dec. 14, 1949.

intermediate point between root and tip, (4) a distributed load varying uniformly from zero at the tip to maximum intensity w at the root, and (5) a constant moment M_k at any intermediate point between root and tip.

In Part I of the dissertation, the shear-lag cases in question are treated as boundary-value problems in the theory of elasticity for isotropic sheets of tension panels and box beams. The conventional engineering theories of bending and direct stress (Mc/I and P/A) are employed to determine stresses in flange members which are subject to bending moments and direct loads whose magnitudes are found from equilibrium and shear-lag requirements. The large-deflection theory in conjunction with strain-energy computations is used in the analysis of buckling.

Tests of two box beams are reported in Part II, and the experimental results are compared with analyses based on the theory of Part I. It was found that maximum flange stresses were obtained within 3 per cent and maximum spanwise sheet stresses within $4\frac{1}{2}$ per cent by means of the theory. Furthermore, it was found that the buckling analysis gave satisfactory results in the calculation of maximum stresses, but small stresses near the free ends of the beams were overestimated. Chordwise sheet stresses were obtained within 6 per cent, except at one point where an error of 14 per cent was observed. From an over-all viewpoint, the theory is considered to be confirmed by the experimental results.

In Appendices A and B, formulas are developed for the evaluation of certain coefficients which occur in the analyses. Detailed numerical analyses of the two test beams are presented in Appendix C. Test data and the stresses calculated from such data are given in Appendix D.

The conclusions determined from the investigation are quoted below.

CONCLUSIONS

The following conclusions are derived from the theoretical and experimental investigations presented herein and are considered applicable at least over the range of length-width (L/b) ratios from 5 to 7 which were included in the box-beam tests.

1. The box-beam analysis is confirmed by the test results.
2. Maximum spanwise stresses in flange members and cover sheets can be calculated within approximately 5 per cent by means of the theory.
3. Chordwise cover-sheet stresses are more affected by fabrication uncertainties and can be calculated with less accuracy than the spanwise stresses.
4. The analysis of compression cover-sheet buckling is suitable for the calculation of maximum stresses.
5. For other than maximum stresses, the buckling analysis is on the conservative side and overestimates the flange stresses.
6. Maximum spanwise stresses in flange members and cover sheets

seriously exceed the stresses computed by means of the conventional bending theory ($\sigma = Mc/I$). For the test specimens, excesses of 10 to 36 per cent were found.

7. For beams with length-width (L/b) ratios comparable to end ratios of the elastic constants $k_a = 2A_s E_s / A_F E_F (1 + \mu) (3 - \mu)$ and $k_b = E_s I_s / E_F I_F (1 + \mu) (3 - \mu)$ smaller than those of the test specimens ($L/b = 5$ to 7 , $k_a = 0.1722$ to 0.2234 , and $k_b = 0.2823$ to 0.3664), maximum spanwise stresses would exceed Mc/I by a higher range of percentages than the range quoted in conclusion 6.

8. For beams with L/b -ratios comparable to and k_a - and k_b -ratios greater than those of the test specimens, maximum spanwise stresses would exceed Mc/I by a lower range of percentages than the range quoted in conclusion 6.

9. Box-beam cover sheets have appreciably reduced effectiveness, if 100 per cent effectiveness is based on the uniform distribution of stress along the width of a beam which is associated with the conventional bending theory. Tension cover sheets were from 91 to 96 per cent effective, and compression covers from 20 to 45 per cent effective in the test specimens.

10. Cover-sheet effectiveness would be less than the percentages quoted in conclusion 9 for beams with L/b -ratios comparable to and k_a - and k_b -ratios less than the test-beam ratios.

11. Cover-sheet effectiveness would be greater than the percentages quoted in conclusion 9 for beams with L/b -ratios comparable to and k_a - and k_b -ratios greater than the test-beam ratios.

12. Coefficients denoted herein as C_1 and C_2 , which are used to satisfy in minor part the boundary condition of equal spanwise displacements of sheet and flange members along the lines where these parts are fastened together, may be neglected without appreciably affecting the results.

THE PROPERTIES OF THE CITRATE COMPLEXES OF THE RARE-EARTH IONS AND THEIR ADSORPTION ON AMBERLITE RESIN¹

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The complexes and water-insoluble compounds of lanthanum, neodymium, and samarium with citric acid, the distribution of lanthanum between citrate solutions and Amberlite resins, and the rate of reaction of lanthanum-chloride solutions with Amberlite IR-100 were investigated.

Absorption spectra of solutions containing neodymium chloride and citric acid at different pH values indicated the existence of four citrate complexes. An absorption band which appeared at $4278.6 \pm 0.5 \text{ \AA}$ was attributed to a H_2cit^- complex of neodymium; a $4286.0 \pm 0.5 \text{ \AA}$ band was attributed to a Hcit^- complex; a $4304.0 \pm 0.5 \text{ \AA}$ band, to a cit^{3-} complex; and a $4312.0 \pm 0.5 \text{ \AA}$ band, to a basic citrate complex.

Spectrophotometric measurements on solutions containing neodymium chloride and citric acid at different pH values showed the effect of pH on the $576\text{-m}\mu$ absorption band of a neodymium chloride solution. In solutions containing citric acid, this band was shifted to longer wavelengths, and the shift was most pronounced in solutions above a pH of 7.

Evidence that a hydroxyl group may enter a citrate complex of lanthanum, of neodymium, and of samarium was obtained by titrating trisodium-citrate solutions of the rare earths with sodium hydroxide.

Other evidence for the citrate complexes was obtained by measuring the distribution of lanthanum between Amberlite resins and citric-acid solutions at various pH values. The resins used were Amberlite IR-100 and Amberlite IR-4; radioactive lanthanum was employed as a means of analysis. In order to explain the experimental data, one had to assume the existence of a H_2cit^- , a Hcit^- , and a cit^{3-} complex of lanthanum. From these data it was calculated that a ratio of one lanthanum to three H_2cit^- existed in one complex; the instability constant was computed to be 6×10^{-7} .

The addition of trisodium citrate to an almost neutral solution of either lanthanum chloride, neodymium chloride, or samarium chloride resulted in the formation of a precipitate and in a decrease in the pH of the solution. After the addition of sufficient trisodium citrate to give a one to one molecular ratio between the rare-earth chloride and the trisodium citrate, an abrupt increase in the pH of the solution occurred, and the precipitate gradually dissolved as more trisodium citrate was

¹ Doctoral thesis number 873, submitted September 22, 1947.

added. These experiments indicated the existence of several different precipitates between each rare earth studied and citric acid solutions. No additional information concerning the nature of these precipitates was obtained from measurements of the refractive index of their filtrates. The analysis of a lanthanum-citrate precipitate which had been dissolved in ammonium hydroxide and re-precipitated with hydrochloric acid, washed with water, and dried at 115°C. corresponded to $\text{La}(\text{C}_6\text{H}_5\text{O}_7) \cdot 2\text{H}_2\text{O}$.

Amberlite IR-100 in the acid cycle was titrated with sodium hydroxide and with ammonium hydroxide. The resin acted as a moderately strong acid, and a break in each titration curve corresponded to 8.58 milliequivalents of hydrogen ion per 5 grams of air-dried resin.

The rate of reaction of a solution of lanthanum chloride with Amberlite IR-100 was dependent upon the pH of the solution and upon the degree of agitation.

The concentrations, corrected and uncorrected for ionic strength, of the three citrate ions in a 5 per cent citric-acid solution at various pH values were calculated.

Procedures were also suggested for the further investigation of the problem.

THE MINERALIZATION OF ORGANIC PHOSPHORUS, NITROGEN, AND CARBON IN VIRGIN AND CULTIVATED SOILS¹

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An investigation was made to determine the relationships of organic phosphorus to nitrogen and carbon in soils and to determine the effect of cultivation on these relationships.

A. Twenty-five pairs of virgin and nearby cultivated soils from Iowa, Texas, and Colorado were analyzed for total nitrogen, organic phosphorus, total phosphorus, and pH.

1. There was a highly significant correlation between total nitrogen and organic phosphorus for both the virgin ($r = 0.726$) and the cultivated ($r = 0.805$) soils.

2. The ratio of nitrogen to organic phosphorus was 11.09:1 for twenty virgin soils and 9.86:1 for twenty cultivated soils of Iowa, which indicates a more rapid decrease of nitrogen than of organic phosphorus during cultivation.

3. The virgin and cultivated soils from Iowa contained averages of 47.4 and 44.8 per cent, respectively, of the total phosphorus in organic form. The Texas and Colorado virgin and cultivated soils contained only 19.8 and 17.1 per cent, respectively, of the total phosphorus in organic form.

4. There was a highly significant negative correlation ($r = -0.465$) between pH and organic phosphorus expressed as percentage of the total phosphorus in the forty soils from Iowa.

5. There was a highly significant correlation ($r = 0.736$) between total nitrogen and total phosphorus in the fifty soils.

6. The twenty cultivated soils from Iowa contained 69.1, 80.0, and 84.2 per cent, respectively, of the original amounts of total nitrogen, organic phosphorus, and total phosphorus. All three of the Texas soils had lost a greater per cent of the original organic phosphorus than of the original total nitrogen, the average percentages lost being 43.1 and 57.7, respectively. Both of the Colorado cultivated soils had lost a greater per cent of nitrogen than of the original organic phosphorus, the values being 12.9 and 8.1, respectively.

B. The mineralization of organic phosphorus, nitrogen, and carbon was determined on three soils incubated for 7 days at temperatures ranging from 10 to 80°C.

1. There was a peak in the curve at 40°C. for phosphorus, nitrogen and, to a lesser extent, for carbon mineralization.

¹ Doctoral thesis number 1043, submitted March 14, 1950.

2. The amounts of phosphorus, nitrogen, and carbon mineralized were much greater in the temperature range of thermophilic organisms than in the range of mesophilic organisms.

3. The peak for nitrogen mineralization was between 60 and 70°C. while the peak for carbon mineralization was at 70°C. The rates of both nitrogen and carbon mineralization dropped markedly at 80°C.

4. The temperature curve for phosphorus showed no peak in the range from 50 to 80°C., and the trend was upward at 80°C.

5. The amounts of organic phosphorus, nitrogen, and carbon mineralized were highly correlated in the biological range of temperature.

C. The amounts of organic phosphorus, nitrogen, and carbon mineralized in twenty-five days at 40°C. were determined for twenty-five pairs of virgin and cultivated soils.

1. The correlation between mineralization of nitrogen and phosphorus was highly significant ($r = 0.807$) for the fifty samples of soil. The regression of phosphorus mineralization on nitrogen mineralization was curvilinear. As nitrogen mineralization decreased, phosphorus mineralization decreased also but to a greater extent.

2. The average ratios of nitrogen to phosphorus mineralized were greater in cultivated soils than in virgin soils. The twenty virgin soils from Iowa released nitrogen and phosphorus in a ratio of 6.31:1 while the twenty cultivated soils released nitrogen and phosphorus in a ratio of 12.53:1. The five virgin soils from Colorado and Texas released nitrogen and phosphorus in a ratio of 9.02:1, while the corresponding cultivated soils released nitrogen and phosphorus in a ratio of 16.17:1.

3. The correlation between mineralization of carbon and phosphorus was highly significant ($r = 0.841$) for the fifty samples of soil. The data fit a curve similar to that for nitrogen and phosphorus mineralization. As carbon mineralization decreased, phosphorus mineralization decreased also but to a greater extent.

4. The average ratios of carbon to phosphorus mineralized were 56.95:1 and 127.47:1, respectively, for the Iowa virgin and cultivated soils, and 157.52 and 161.55, respectively, for the virgin and cultivated soils from Texas and Colorado.

5. The highest correlation ($r = 0.880$) was found between the mineralization of nitrogen and carbon. The regression was linear.

6. The average ratios of carbon to nitrogen mineralized were 9.57:1 and 10.29:1, respectively, for the Iowa virgin and cultivated soils.

7. The virgin soils from Iowa mineralized averages of 7.97 and 3.97 per cent of their organic phosphorus and total nitrogen respectively. Corresponding results for the cultivated soils were 3.52 and 3.17 per cent. These data indicate that virgin soils contain a source of readily mineralizable organic phosphorus which decreases very rapidly on being brought into cultivation.

THE ANTHRACNOSE COMPLEX ON SOYBEANS¹

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The legumes commonly grown in Iowa are all hosts to one or more of the anthracnose fungi. Except for the northern anthracnose of red clover which was not included in these studies, anthracnose on this group of hosts is caused by various species of *Colletotrichum*. On soybeans, one of the relatively new legumes which is taking an important part in midwest agriculture, anthracnose is one of several diseases which is considered to be of minor importance at the present time. It is potentially important, however, for as extensive plant breeding work is carried on with a wide range of host plant material, diseases that are responsible for very little loss in plantings of the present standard varieties may become quite important.

Three anthracnose fungi, *Colletotrichum truncatum* (Schw.) Andrus and Moore, *Colletotrichum destructivum* O'Gara, and *Glomerella glycines* (Hori) Lehman and Wolf commonly occur on soybeans. *Colletotrichum truncatum*, the form originally discussed as the causal agent of soybean anthracnose, was found to be the most active parasite of the group. When plants of the variety Lincoln were spray inoculated in the field with a suspension of conidia of this species, the fungus was recovered from leaves that did not exhibit any symptoms of disease.

Seed borne inoculum of *C. truncatum* was responsible for several types of infection. They were: (1) pre-emergence killing, (2) seedling blight, (3) symptomless establishment of internal mycelium. The third type was investigated in detail. Mycelium from infected cotyledons became established in the cortical cells of the stem without any apparent effect upon them and remained localized in the immediate stem area originally entered until the time of flowering. It then resumed growth and penetrated cells in the stem, petioles, and leaves without the development of disease symptoms. When pods started to develop after fertilization, mycelium was found in the cells of the pod. These pods and the developing seeds also exhibited no evidence of disease.

The anthracnose fungi that occur on other common legumes were examined and cross inoculation studies were also conducted with them. Three curved spore forms, *C. truncatum*, *C. graminicolum* (Cesati) Wils. and *C. villosum* Weimer and four species with straight conidia, *C. lindemuthianum* (Sacc. and Magn.) Briosi and Cavara, *C. trifolii* Bain and Essary, *C. destructivum*, and *Glomerella glycines* were included. As a result of these comparisons, the entities themselves were more clearly defined and their host ranges extended.

¹ Doctoral thesis number 1066, submitted June 3, 1950.

Red clover was attacked by *C. destructivum*, *C. trifolii*, *C. truncatum*, and *C. graminicolum*. Of these, the first two are the fungi usually responsible for anthracnose on this host. Soybeans, alfalfa, and sweet clover were also susceptible to the same four fungi. On vetch, *C. villosum* and *C. truncatum* were capable of causing disease. Garden beans were attacked successfully only by *C. lindemuthianum*. No inoculations were attempted on grasses, although isolates from alfalfa and sweet clover have been considered to belong to *C. graminicolum*, a species that at the present time includes a complex of curved spore forms described from a wide range of grass hosts.

Some members of this group of anthracnose fungi have been connected with a *Glomerella* as the ascogenous stage. The only perithecial form examined was *Glomerella glycines* which occurs on mature soybean stems. Its conidial phase is not *Colletotrichum glycines*, but is a straight spored form similar to *C. destructivum*.

EFFECT OF CERTAIN ORGANIC AGENTS ON WATER-STABLE SOIL AGGREGATION ¹

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The extent and the stability of soil aggregation is of the greatest importance in agronomy. Good, *i.e.* water-stable, aggregation decreases erosion, increases infiltration capacity, friability and aeration. Part of the beneficial effect on aggregation caused by cropping soils in grasses and legumes is due to the increase or maintenance of the soil organic matter. Water-stability of soil aggregates might therefore conceivably be influenced by relatively small amounts of certain organic compounds.

In a review of the literature it is shown that several investigators have been able to influence the water-stability of soil aggregates markedly by small additions of organic compounds. In some cases the influence exerted was related to base exchange phenomena of the soil with large organic cations of which the active group is an amino group. In other cases the increase of water-stability was affected by such compounds as resins, fats, waxes, and bacterial polysaccharides. In such cases the cause of the effect seemed to be a modification of the wettability of the soil, although this was not explicitly shown.

In the present investigation a new technique was proposed to comprise in a single figure the results of an aggregate analysis by wet sieving, in order to evaluate more clearly and comprehensively the effects of certain treatments on the aggregation of the soil. The data obtained were plotted as an accumulative percentage curve and the area between such a curve and the 100 per cent line (percentages being plotted on the ordinate) was measured. It was shown that this area is equal to the mean diameter, which, since the frequencies found were based on weights, was termed the mean weight-diameter. By several examples it was substantiated that the proposed index of water-stable soil aggregation was consistent and satisfactorily accurate.

A description was given of the preparation of a hydrochinon polymer, that has received some attention in the literature due to its resemblance to soil organic matter. A dark-colored powder was obtained, forming dark-colored colloidal alkaline solutions and having an exchange capacity of 330 m.e. per 100 g. Addition of this polymer in colloidal solution to puddled soil did increase the aggregation somewhat, but it actually decreased the aggregation of soils in natural condition.

Following previous work of other investigators the effect of ethylamine, butylamine, and benzylamine in aqueous solutions on aggregate

¹ Doctoral thesis number 1015, submitted December 12, 1949.

stability was investigated. Addition of amounts of the listed amines equivalent to 10 per cent of the total base exchange capacity of the soil revealed that benzylamine decreased markedly the turbidity of soil suspensions, in contrast with ethylamine and butylamine that had a small effect only. Soil aggregation was increased 140 per cent, as measured by the mean weight-diameter, by treatment of soil with benzylamine, whereas ethylamine and butylamine gave increases of 65 per cent and 32 per cent respectively. In the latter cases amounts equivalent to 30 per cent of the total exchange capacity of the soil were added to the soil. It was noted that the dry strength of soil aggregates was almost nil as a result of the latter treatment, especially the one with benzylamine, and it was concluded that this fact would seriously decrease any practical significance of soil treatment with amines.

The influence of modification of soil wettability on aggregate stability was studied by treating air-dry soil, containing 3 to 4 per cent moisture, with methylchlorosilanes. These compounds will hydrolyze readily with the water in the soil and form a polymerized organo-silicon deposit that is very water repellent. Treatment of the soil was done by fumigation at reduced pressures at rates of 0.2 per cent and 0.5 per cent by weight. It was found in a number of tests that the aggregate stability was increased very markedly. As measured by the mean weight-diameter, increases of 600 per cent and more could be obtained at the highest rate used. As an index of the influence of the same treatment on the engineering properties of soils and soil materials, the changes in plastic index induced by treatment were studied. Reductions of the plastic index of soils of the order of 15 per cent were obtained, which would indicate an appreciable improvement of the suitability of the treated soils for construction purposes.

Pertinent investigations were made to study the effect of treatment of air-dry soil with methylchlorosilanes at rates of 0.2 per cent and 0.5 per cent by weight on soil properties other than aggregation. It was found that moisture equivalent and hygroscopicity were almost unaffected. A slight increase in acidity of approximately half a unit in the pH scale and an increase of the total base exchange capacity by several per cents were noted. To study the possible antibiotic effects of the treatment CO_2 -evolution of treated and untreated soil supplied with some energy material was studied in a preliminary experiment and no effect found. More experiments will be needed to clarify this particular point.

In order to obtain more understanding about the nature of the reaction involved, H-bentonite was treated in a manner similar to soils as described above. Thermal analysis of the product tended to indicate that the hydrolysis product is not in any way chemically combined with the clay or a part of the crystal lattice. The organo-silicon deposit burns off at low temperatures ($150^\circ\text{C}.$) and seems therefore present at the outer edges of clay aggregates. This conclusion was also substantiated by X-ray diagrams taken of treated clay. Even at high rates of

treatment (16 per cent by weight) no trace could be found of any change in the principal lattice constants of the clay.

The general conclusion was drawn that treatment of soil with methylchlorosilanes is a convenient method to increase the water-stable aggregation significantly. The possibility of treating soil with warm, dry air in which methylchlorosilanes were volatilized at normal pressure was investigated and found feasible. It was furthermore concluded that the reaction studied could be the basis for an important practical application in agronomy, in agricultural engineering, and in soil mechanics.

ELECTROLYTIC CONCENTRATION OF CAUSTIC SODA¹

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In the commercial production of electrolytic caustic soda it is necessary to concentrate the effluent from the chlorine-caustic cell in order to produce a caustic of a marketable concentration. At the present time, the effluent is concentrated by means of multiple-effect evaporators, usually to a 50 per cent caustic soda solution. In this work a process was proposed and studied in which the effluent from a chlorine-caustic cell was concentrated in a continuous manner to a 50 per cent caustic soda solution by electrolysis of the water in the solution. Considerable quantities of hydrogen and oxygen are produced as by-products.

Since the effluent from a chlorine-caustic cell contains not only about 11 per cent sodium hydroxide, but also from 14 to 18 per cent sodium chloride, it was necessary to investigate the factors affecting the tendency for chlorine evolution when this solution is subjected to electrolysis; and then to find a method of preventing this evolution of chlorine. The tendency for chlorine evolution is controlled by the electrode material and its surface characteristics, the current density, the salt concentration, the temperature, and the electrode spacing. Tests were carried out on nickel and monel metal electrodes. The tendency for chlorine evolution is enhanced by a high current density and a large electrode spacing. The use of an elevated solution temperature and smooth electrodes decreases the tendency for chlorine evolution.

A small circular electrode cell was built and operated in a batch manner; but it was unsuccessful due to difficulties caused by chlorine evolution. A small parallel electrode cell was constructed and operated in a batch manner, but again difficulties were caused by chlorine evolution. This cell was modified so as to make its operation continuous. It was demonstrated that it is possible to concentrate, in a continuous manner, the effluent from a chlorine-caustic cell to a solution containing 50 per cent caustic soda. The salt of the feed solution precipitated during the process.

Studies were carried out on the factors affecting the cell voltage when electrolyzing a caustic-salt solution. Variables studied were solution concentration, electrode spacing, temperature, and current density. Tests were made with both monel metal and nickel electrodes. It was found that the overvoltage was slightly less for the nickel than for the monel metal. Stainless steel was tried as a cathode, but its overvoltage

¹ Doctoral thesis number 1054, submitted May 9, 1950.

was found to be much higher than that of nickel. The results of all the conductivity tests are presented.

A pilot plant concentration cell was built and operated. A 30-hour continuous test demonstrated its practicability, during which time an effluent from a chlorine-caustic cell was continuously concentrated to a 50 per cent caustic solution. The effect of temperature and current density on the pilot plant cell voltage was investigated. Studies were made on the amount of caustic and water removed with the hydrogen and oxygen. It was found that entrainment was not excessive, but that from 14 to 21 per cent of the water removed during concentration was removed as water vapor in the gases.

On the basis of data obtained from the pilot plant cell, a commercial-sized concentration cell was designed and its cost was estimated. An economic evaluation was made for a plant utilizing the electrolytic concentration process having a capacity of 50 tons of caustic soda per day produced as a 50 per cent solution. The plant will also produce about 43 tons of chlorine per day, $2\frac{1}{2}$ tons of hydrogen per day, and 197 tons of oxygen per day. Since the concentration cell-current density affects both the investment cost and the power cost, a complete study was made for each of four concentration cell-current densities. The most economical current density was 150 amperes per square foot. Under these conditions, a fixed capital investment of \$4,975,000 is required. Of this, \$1,246,000 is for the concentration cells, and \$1,140,000 is for the rectifier. A working capital of \$440,000 is required, making a total capital investment of \$5,415,000. If power is available at three mills per kilowatt-hour, the yearly production cost is \$2,668,000. If 50 per cent caustic is sold for \$48 per ton of NaOH, chlorine for \$48 per ton, cylinder oxygen (4.4 tons per day) for \$8 per ton, and hydrogen for 50 cents per thousand cubic feet, an annual income of \$3,900,000 is realized. After deducting sales, administrative, and research expense estimated as 5 per cent of the annual sales, and 43 per cent of the net profit before taxes for federal and state income taxes, a net profit of \$592,000 is obtained. This gives a return on the investment of 10.9 per cent.

Possible applications of the proposed process in the soap industry and in the heavy chemical industry are discussed. Recommendations for future work on cell design are made.

COMMUNITY SERVICES OF VOCATIONAL AGRICULTURE GROUPS IN THE NORTH CENTRAL AND WESTERN REGIONS¹

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Community services have been a part of the program in vocational agriculture since the passage of the Smith-Hughes Act in 1917. It is assumed that community services will continue to be a part of the program of many departments of vocational agriculture. The purpose of the present study was to formulate techniques for selecting and conducting community services of the vocational agriculture groups. These groups are the all-day, young farmer, adult farmer, and veterans' groups.

Community services of vocational agriculture groups include activities of the groups in performing services for members and in many cases for others in the community. Some of the community services are activities performed through the classes and some are performed through organizations of members of the classes.

The method of procedure in the present study was planned to obtain information which would assist in the development of techniques for selecting and conducting community services. Two generally accepted methods of determining techniques were used in the present study; first, a survey of present practices was made; and second, recommendations of authorities were obtained. Teachers of vocational agriculture, supervisors, teacher trainers, and superintendents, as well as lay participants in the services, cooperated in the present study. Ten of the community services which were among the most frequently reported in the *Agricultural Education Magazine* from 1932 to 1946 were selected for study.

Schedules which included questions concerning attitudes toward community services were sent to all teacher trainers and supervisors of vocational agriculture in the North Central and Western regions. Identical schedules were sent to fifty-one superintendents of school. Usable returns were received from 144, or 96 per cent, of the persons to whom schedules were sent. Schedules in the form of return postal cards were sent to all teachers of vocational agriculture in twenty-three states of the twenty-four in the two regions on which the teachers reported the status of the services in their programs. Returns were received from 1,372, or 50 per cent of the teachers to whom schedules were sent. Schedules on which the teachers could report more fully concerning the repair shop, cannery, and soil conservation services were sent to teachers who reported on the return postal cards that they had one or more of these three services in their program. Schedules were returned

¹ Doctoral thesis number 1016, submitted December 13, 1949.

for 1,031 services. Schedules were sent to 190 participants of the services on which they were asked to report attitudes toward community services. Of this number, ninety-seven were returned. The data thus obtained were used as a basis for the development of techniques for selecting and conducting the community services of vocational agriculture groups.

The ten services selected for general study were divided into three groups. The cooperative ownership services included livestock association, cooperative buying, cooperative marketing, and machinery cooperative services. The cooperative nonownership services included dairy herd improvement, seed treatment, seed testing, and soil conservation services. The work-center services included the repair shop and community cannery services. In discussions of the regions, Kentucky and Missouri were considered as one region. The remainder of the states in the North Central Region were regarded as another region and the states of the Western Region as a third region. The supervisors, teacher trainers, and superintendents were defined as administrators for the purpose of the present study.

The first problem undertaken in the present study was the examination of certain factors concerning the selection of community services of vocational agriculture groups. Information was obtained from the teachers concerning the value of nine methods which had been used for the selection of community services. Selection on the basis of the decision of the vocational agriculture groups was rated as the best method of selecting services. The other methods considered were selection on the basis of community survey, requests of individuals, recommendation of advisory council, decision of the teacher, special survey, request of organizations, advice of supervisory staff, and decision of school authorities. More teachers rated each method as an excellent method and as a fair method than rated any method as poor. Thus, each method was recognized as having value in the selection of community services.

Information was obtained in regard to the adaptability of community services in the three regions, adaptability to size of town, adaptability to size of school, and adaptability to the vocational agriculture groups. The relative adaptability was determined by the number of services which had been organized, the continuance of the services, and the accomplishments in the services. The cooperative ownership services were most adaptable to the Kentucky-Missouri and Western regions, to the large towns, and to schools of large or medium size. Cooperative nonownership services were most adaptable to the North Central Region, to the towns of medium size, and to schools of medium size. Work-center services differed in their adaptability. Repair shop services were most adaptable to the Western Region, to small towns, and to schools of medium size. Community cannery services were most adaptable to the Kentucky-Missouri Region, to large towns, and to large schools.

The adaptability of repair shop, community cannery, and soil conservation services to the vocational agriculture groups was determined.

The adaptability was based on the number of services and the number of participants in the services. Repair shop services were more adaptable to all of the vocational agriculture groups than were the other services. Repair shop and soil conservation services were adaptable to all groups, whereas community cannery services were adaptable to adult groups only.

Information was obtained also in regard to the relationships of community services to private business. The teachers reported that in general people in private business were cooperative with community service programs. According to the teachers, one-half of the services concerning which reports were received competed to some extent with private business. Seventy-eight per cent of the teachers, administrators, and participants who responded believed that competition was all right. The foregoing facts suggest that attention should be given to the development of rapport with private business.

According to the respondents of the present study, the most beneficial outcome of the cooperative ownership services was the development of a cooperative attitude. The most beneficial outcome of the cooperative nonownership services was increasing the income of the participants. Beneficial outcomes of the work-center services varied greatly. Improvement of home living was the most beneficial outcome of community cannery services, and improvement of farm efficiency was the foremost outcome of repair shop services.

The second problem of the present study was the examination of certain factors concerning the conducting of community services. Information was obtained from the teachers in regard to the degree of value of nine factors contributing to the success of community services. Seventy-nine per cent of the teachers indicated that the fact that the services fulfilled a need was a major reason for the success of community services. No teacher indicated that this factor had been of no value. More teachers rated each of the nine factors as of major value and as of some value in the success of community services than rated any factor as of no value. All of the factors were recognized by a large majority of the teachers as important in the success of community services.

Information was obtained with respect to the use of systematic instruction in the case of 1,031 repair shop, community cannery, and soil conservation services. Systematic instruction was given in connection with 47 per cent of these three services. Many of the respondents stated that education of the participants in the services is the only reason for the community service programs of vocational agriculture groups. Not a single respondent stated that the community services should not have systematic instruction in connection with the services.

Special instructors were employed in 41 per cent of the repair shop, community cannery, and soil conservation services which were reported. More special instructors were employed in community cannery services than in the other services. More special instructors were employed in the services of veterans than in the services of other vocational agricul-

ture groups. Since the teacher of vocational agriculture is trained to give instruction in most community services there are many which do not need special instructors. The need arises when the service requires special skills for which the teacher is not trained and also when the community service program expands to such an extent that it takes too large a portion of the teacher's time.

Current expenses were considered in the present study as those expenses of a service other than instruction, permanent equipment, and housing. Forty-two per cent of the services concerning which information was obtained were self-supporting for current expenses. More of the services of the adult group were self-supporting than of the other vocational agriculture groups. More of the community cannery services were self-supporting than was true of the other services. Many of the respondents of the present study maintained that all community services should be self-supporting. None of the respondents expressed belief that any of the services should not be self-supporting.

Seventy-five per cent of the administrators who responded indicated that the Future Farmers can help with cooperative nonownership services. It may be concluded that these are services with which the Future Farmers should help. Two-thirds of the administrators reported that the Future Farmers can help with cooperative ownership services. Apparently Future Farmers may be helpful with these services. Less than two-thirds of the administrators believed that the Future Farmers can help with work-center services. Hence, it would appear that the effectiveness of Future Farmers' help with these services is questionable. The Future Farmers assisted in a majority of the services reported in the *Agricultural Education Magazine*.

A majority of the administrators and teachers who responded believed that community services should be aided indefinitely. However, over one-third of the administrators, one-fourth of the teachers, and three-fourths of the participants of the services reported that the services should not be aided indefinitely. Education should be carried on as long as it is needed, but it would seem that when the services become capable of self-sustenance there should be provision for the service to become independent of the official school groups.

Based on the findings in the present study, techniques were developed for use by the teacher of vocational agriculture in selecting and conducting community services.

TECHNIQUE FOR SELECTING COMMUNITY SERVICES

- Step 1 - Determine the services which are adaptable to the region.
- Step 2 - Determine the services which are adaptable to the size of town.
- Step 3 - Determine the services which are adaptable to the size of school.
- Step 4 - Determine the services which are adaptable to the vocational agriculture groups.
- Step 5 - Determine the services which will give the beneficial outcomes desired.
- Step 6 - Develop rapport with private business.
- Step 7 - Make a community survey.

- Step 8 - Discuss the community service program with individuals, with representatives of local organizations in the community, and with members of the supervisory staff.
- Step 9 - Present information concerning community services to the advisory council for recommendations.
- Step 10 - Present the information concerning community services to the vocational agriculture groups for their decision.
- Step 11 - Present the community service program to the school authorities for their approval or disapproval.

A teacher of vocational agriculture should find it helpful to go through the foregoing steps during his first year in a new school. It should be helpful also to go through the steps for any new community service being considered.

TECHNIQUE FOR CONDUCTING COMMUNITY SERVICES

- Step 1 - Formulate a definite plan of systematic instruction for all of the participants in order to teach the skills and abilities involved in the service.
- Step 2 - Provide for a special instructor if the type of the service or the size of the program demands such an instructor.
- Step 3 - Make definite financial plans for current expenses.
- Step 4 - Provide an opportunity for the Future Farmers to help with the community service.
- Step 5 - Provide in the organization of the service a procedure by which the service may become independent of the vocational agriculture department if this plan is later deemed advisable.
- Step 6 - Organize the service in such a way that the participants may become the leaders.
- Step 7 - Provide adequate facilities for the service.
- Step 8 - Formulate and carry on a systematic publicity program.
- Step 9 - Formulate a plan for reporting new and improved practices which are adopted.
- Step 10 - Evaluate the program, ascertaining whether the service fulfills a need.

A teacher of vocational agriculture should find it helpful to go through the foregoing steps for each community service before finally starting the service. The steps should be reviewed occasionally throughout the time that the service is in operation.

ENZYMIC PEPTIDE BOND SYNTHESIS AND ITS INHIBITION¹

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The investigations reported in this thesis were concerned with the preparation of a number of acylamino acids and the determination of the conditions influencing their abilities to undergo peptide bond (anilide) synthesis as catalyzed by the proteolytic enzyme, papain. The factors investigated included: variation of the nature of the acyl group (benzoyl, *p*-nitrobenzoyl, carbobenzoxy, and carboallyloxy), variation in the nature of the amino acid residue (glycine, DL-valine, DL-leucine, and L-glutamic acid), variation of the pH (from ca. 3.0 – 6.5) and variation of the citrate buffer concentration (1.0 *M* and 0.1 *M*). The extents of hydrolysis of the amides of benzoylglycine, benzoyl-DL-valine and benzoyl-DL-leucine by papain at pH 5.0 in 1.0 *M* and 0.1 *M* citrate buffers were also determined.

The efficacies of various substances as inhibitors of the papain-catalyzed syntheses of carbobenzoxy-L-glutamic acid anilide and benzoyl-L-leucine anilide at the pH optima of the syntheses, and in 1.0 *M* citrate buffers have been tested. Various group reagents were employed in attempts to determine the essentiality of certain groups of the enzyme for its synthetic ability.

The pH optima for the syntheses of all the acyl-L-glutamic acid anilides were lower, in both 1.0 *M* and 0.1 *M* citrate buffers than the optima for the anilides of the corresponding acyl derivatives of glycine, L-valine and L-leucine. The former values were in the range of pH 4.1 to 5.0.

With but few exceptions the pH optima for the acyl derivatives of the monoaminomonocarboxylic acids increased in the order: acylglycine, acyl-DL-valine and acyl-DL-leucine. These values fell in the range of pH 4.2 to 6.5.

The pH optima for the syntheses of all the acylamino acid anilides were from 0.2 to 0.9 pH units lower in the 0.1 *M* buffers than the corresponding values in the 1.0 *M* buffers.

The yields of the anilides when 1.0 *M* buffers were employed were, in almost all instances, greater than the yields of the same compounds in 0.1 *M* buffers at the pH optima of the reactions.

Carboallyloxy-L-glutamic acid was the only acylamino acid studied which failed to yield an anilide in either 1.0 *M* or 0.1 *M* buffer solutions.

¹Doctoral thesis number 1001, submitted September 14, 1949.

Carboallyloxyglycine and carboallyloxy-DL-valine did not give measurable yields of anilides in 0.1 *M* citrate buffers.

Although variations in the nature of acyl groups attached to any one amino acid residue resulted in changes in the pH optima of the synthetic reactions, the orders of the changes were not the same for all the amino acids studied.

The yields of the anilides obtained by altering the nature of the acyl group attached to any one amino acid residue also varied with these changes. However the effects were not too pronounced and, in the main, there was a greater dependency on the nature of the amino acid than on the nature of the acyl group; no definite order in the yields was noted with variation of the acyl groups.

The orders of the yields of the anilides of the acylated monoaminomonocarboxylic acids in both 1.0 *M* and 0.1 *M* buffers were in all cases acyl-L-leucine > acylglycine > acyl-L-valine. The acyl derivatives of glutamic acid which gave insoluble products, showed yields of their respective anilides comparable to those of the corresponding leucine derivatives. Such results indicated that variation in the nature of the amino acid residue altered the reactivities of the substrates in a definite manner and to a greater extent than did changes in the acyl groups. The results supported the view that an enzymic preference for the amino acid residues existed.

At pH 5.0 in both 1.0 *M* and 0.1 *M* citrate buffers, benzoyl-DL-leucinamide was hydrolyzed to a greater extent by papain than was benzoyl-DL-valinamide. These results, when correlated with those of the synthetic experiments, indicated a preference by papain for leucine residues relative to valine residues.

The extents of inhibition of the papain-catalyzed syntheses of carbobenzoxy-L-glutamic acid anilide and benzoyl-L-leucinanilide by a number of substances varied with either the nature of the acylamino acid substrate or the pH and the time of the synthetic reaction.

The degrees of inhibition were apparently independent of either the nature of the substrate or the pH when copper sulfate, sodium bisulfite, hydroxylamine, nitrous acid, formaldehyde, and potassium ferricyanide were used as inhibitors. *p*-Benzoquinone inhibited both reactions weakly and to the same extent when the enzyme was not pretreated with this compound. Pretreatment of papain with the quinone resulted in a greater degree of inhibition of the synthesis of the acylleucinanilide, but did not markedly change the extent of formation of the glutamic acid derivative.

The extent of inhibition of each of the two synthetic reactions was dependent on either the nature of the substrate or the pH in the cases of phenacyl bromide, maleic acid, fumaric acid, 2-methyl-1,4-naphthoquinone bisulfite, 2,3,5-triphenyltetrazolium chloride, and 2,5-diphenyl-3-(*p*-iodophenyl)-tetrazolium chloride. These compounds inhibited the synthesis of carbobenzoxy-L-glutamic acid to a greater degree than that of benzoyl-L-leucinanilide. Converse results were obtained with *p*-

iodoaniline, basic phenylmercuric nitrate and 2-hydroxy-5-methylacrylophenone. Possible reasons for these differences have been discussed.

The papain-catalyzed syntheses were inhibited by several sulfhydryl reagents (iodoacetic acid, phenacyl bromide, basic phenylmercuric nitrate, maleic acid, and oxidizing agents). Such results thus indicated that sulfhydryl reagents inhibit the synthetic activities of papain as well as the proteolytic abilities as previously shown.

Studies conducted with carbonyl reagents gave results which when compared with previous observations showed that these reagents were capable of inhibiting both the synthesis and hydrolysis of peptide bonds.

During the course of the investigation the following compounds previously not reported in the literature were prepared: carbobenzoxy-DL-valine, m.p. 76–78° (first time analyzed); carbobenzoxy-DL-leucine, m.p. 45–48° (first time analyzed); carboallyloxy-DL-valine, m.p. 49.5–52°; carboallyloxy-L-glutamic acid, m.p. 55–58°; benzoyl-DL-valine ethyl ester, m.p. 65–68°; carbobenzoxyglycine ethyl ester, m.p. 33–34°; carbobenzoxy-DL-valine ethyl ester, m.p. 32–33°; carbobenzoxy-DL-leucine ethyl ester, m.p. 18.5–19°; carboallyloxy-DL-valine ethyl ester, m.p. 9–11°; carbobenzoxyglycinamide, m.p. 136–137.5°; carboallyloxyglycinamide, m.p. 107–107.5°; carboallyloxy-DL-leucinamide, m.p. 83–85°; benzoyl-L-glutamic acid anilide, m.p. 169–171°; *p*-nitrobenzoylglycinanilide, m.p. 213.5–215.5°; *p*-nitrobenzoyl-L-valinanilide, m.p. 215–216°; *p*-nitrobenzoyl-L-leucinanilide, m.p. 188–190°; *p*-nitrobenzoyl-L-glutamic acid anilide, m.p. 191–192°; carbobenzoxy-L-valinanilide, m.p. 182–183.5°; carbobenzoxy-L-leucinanilide, m.p. 138–141° (first reported melting point); carboallyloxyglycinanilide, m.p. 134–136°; carboallyloxy-L-valinanilide, m.p. 168–169°; and carboallyloxy-L-leucinanilide, m.p. 160.5–162°.

CHARACTERIZATION OF BACTERIOPHAGES ACTIVE AGAINST LACTIC STREPTOCOCCI¹

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An investigation was undertaken to characterize, especially from a taxonomic standpoint, a series of sixty-six bacteriophage preparations active against the lactic streptococci, *Streptococcus lactis* and *Streptococcus cremoris*. Of these preparations, forty-two were obtained in the United States, principally in Iowa, and twelve were received from New Zealand, ten from England, and two from Canada. The characteristics considered were (1) serological grouping, (2) cross-reactions of the bacteriophages and host cultures, (3) heat inactivation, and (4) plaque sizes. Data on morphology were available from other sources.

The bacteriophages were inoculated into susceptible cultures growing in skim milk, and after incubation for 8 hours at 32°C. the material was coagulated by acidification. Bacteria-free whey filtrates were prepared, and the titers were determined in litmus milk using a three-tube limiting dilution method, the concentration of active bacteriophage particles being calculated from probability tables.

For preparation of antiphage sera various whey filtrates were adjusted to pH 7.4 and injected intraperitoneally into rabbits at 48-hour intervals for about one month, starting with small amounts and gradually increasing up to 5 ml. per dose. The sera obtained were preserved by freezing and diluted 1:10 with distilled water for use in demonstrating the phage-antiphage reaction. Bacteriophage filtrates diluted 1:10 in skim milk were mixed with an equal amount of diluted serum and incubated at least 1 and not more than 4 hours at 35°C. The decrease in bacteriophage titer was used as a measure of antiphage activity. Titer decreases when homologous bacteriophage and serum were used were in the order of 10^6 to 10^8 , showing the bacteriophage to be highly antigenic. Cross-neutralization reactions were carried out using twelve sera against the bacteriophage strains used to prepare the sera. When one serum neutralized its homologous bacteriophage but did not exhibit cross-neutralization with other bacteriophage strains, such differentiation was used to identify several serological groups. When several related sera exhibited reciprocal cross-neutralization by reacting with their several homologous bacteriophage strains, those strains were considered members of the same serological group. The different patterns exhibited by the twelve sera were shown to represent seven serological groups. In some cases one bacteriophage strain was neutralized by one or more

¹ Doctoral thesis number 1010, submitted November 30, 1949.

apparently unrelated sera, while the bacteriophages used to produce these sera were not, in turn, neutralized by the antiserum of the first strain. These reactions suggested that certain bacteriophage strains have partial common antigenic characteristics which prevent complete separation of the various bacteriophage strains into distinct serological groups. Techniques for segregation of possible common antigenic components were not employed.

Cross-neutralization reactions were carried out using the twelve sera against the sixty-six available bacteriophage strains. The resultant patterns were used to establish the group relationships of the various strains. Some borderline cases limited somewhat the reliability of the method employed, but the serological patterns permitted differentiation into apparently reasonably homogeneous groups. It was possible to classify fifty-one strains into seven groups, while fifteen strains were not neutralized by any of the twelve available sera.

For the cross-reaction studies, one drop each of bacteriophage and culture was added to a tube of litmus milk and examined after incubation to determine whether the bacteriophage had shown any inhibitory effect on the organism. Inability to give consistent patterns and the general tendency toward strain specificity have limited the usefulness of the cross-reaction method for classification. However, when cross-reactions of sixty-six bacteriophage strains and forty-nine cultures were carried out under good conditions, which included nutritional fortification of the skimmilk with vegetable juice, the various bacteriophage strains exhibited activity patterns somewhat characteristic for the seven serological groups.

For the heat inactivation studies whey filtrates were diluted 1:100 in skimmilk and the reaction was adjusted to desired pH levels. The final concentration was greater than 10^6 bacteriophage particles per milliliter. Appropriate numbers of 10 ml. quantities of material in screw cap tubes were submerged in constant-temperature water baths for heating and quickly cooled at the end of the desired time intervals. Measurement of surviving active bacteriophage particles showed that the inactivation of the bacteriophage progressed rapidly during the initial stages of heat treatment, but the presence of some more heat-resistant particles in the population prevented a linear relationship between the logarithm of survivors and the time of heat treatment. The maximum heat resistances of 18 representative strains at pH 7.0 in skimmilk were compared.

Complete inactivation for the different strains was obtained with conditions ranging from 75°C. for 12 minutes down to exposures no more rigorous than pasteurization. Representative strains of a group based upon serological and cross-reaction results had similar levels of heat resistance. The various strains could be differentiated on the basis of high, medium, or low resistance; such grouping in a general way agreed with the groups established by serological and cross-reaction determinations.

Representative bacteriophage strains were plated on susceptible

host cultures, and the diameters of the resultant plaques were measured for comparative purposes. Except for one strain (F24), which produced very small plaques (about 0.33 mm.), the median diameters ranged from 1.1 to 1.6 mm. Since all were approximately the same general size, no correlation of plaque size with other characteristics of the various bacteriophages was apparent, except that the strain producing the unusually small plaques also was quite readily inactivated by heat.

Electron micrographs from other sources showed bacteriophage particles of representative strains of the seven groups so nearly alike in size and shape that they could not be differentiated on the basis of morphology.

For classification purposes, the serological groupings were given primary consideration, and cross-reaction results were used for confirmation of the seven groups established. The serological method did not show as many inconsistencies as the cross-reaction method. Heat inactivation and plaque size information served to identify more completely the respective groups. These four methods of characterization provided a basis for a systematic classification of fifty-one of the sixty-six bacteriophage strains active against the lactic streptococci which were studied.

PROTEOLYTIC ENZYMES OF RIPENING CHEDDAR CHEESE¹

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The changes induced by the proteolytic and lipolytic enzymes to be found in cheese are considered to be the important phenomena in the ripening process of cheddar cheese. Proteolytic enzymes contained in the cheese are responsible for the progressive hydrolysis of cheese protein which results in a gradual softening or breaking-down of the cheese body. These proteolytic changes also are rather closely related to the development of flavor in the cheese. At the present time it is quite generally thought that proteolysis of cheddar cheese comes about as a result of the action of the rennet extract added in the manufacturing process and through the action of proteolytic enzymes associated with the bacteria contained in the cheese. The purpose of this investigation was to determine more exactly the relative importance of rennet extract on the ripening process of cheddar cheese and to investigate the proteolytic enzyme system of *Streptococcus lactis* in relation to cheese ripening.

Nine series of cheese were manufactured from pasteurized milk employing quantities of rennet extract of from 1.5 to 8 ounces per 1,000 pounds of milk. The analyses of these cheese indicated that soluble nitrogen production in the cheese increased as the quantity of rennet extract employed was increased. It was shown that rennet extract in the concentration in which it normally is used in cheese was responsible for a considerable portion of the hydrolysis of cheese protein during the ripening period. In a 180-day ripening period the flavor scores of cheese made with 4, 6, and 8 ounces of rennet extract were equal to, or better than, those of cheese made with 2 ounces of rennet extract. In three series of cheese ripened at 43° and 50°F., the body and texture of the cheese made with 4, 6, and 8 ounces of rennet extract was as good as that made with 2 ounces of rennet extract.

The results obtained when cheddar cheese was manufactured with different quantities of rennet extract indicated that the rennet extract was of some help in obtaining faster ripening cheese. However, rennet extract alone was not the answer to the problem.

Cheddar cheese was manufactured from pasteurized milk employing equal milk-coagulating quantities of commercial rennet powder and rennin prepared according to the directions of Hankinson. There were no consistent differences in soluble nitrogen production or in the flavor, body, and texture characteristics among the cheese manufactured with these enzyme preparations.

¹ Doctoral thesis number 1040, submitted March 13, 1950.

The milk-coagulating activities of rennet extract, rennet powders, and rennin were determined on total nitrogen and salt-free dry weight bases. The rennin preparation tested was approximately thirteen times as active as the rennet extract from which it was obtained on a unit weight of nitrogen basis and about ten times as active as the rennet extract on a salt-free dry weight basis. The rennet powders were six to seven times as active as rennet extract per unit weight of nitrogen. The commercial rennet powder had about the same activity as rennet extract on a dry weight basis, while the special rennet powder was about five times as active as rennet extract on a salt-free dry weight basis.

Measurements of proteolysis by rennet extract, rennet powders and rennin were made on hemoglobin and casein, using essentially the method of Anson. This method measures tyrosine and tryptophane colorimetrically in the solution remaining after the precipitation with trichloroacetic acid of the unchanged protein in the enzyme-substrate reaction mixture. Maximum values for proteinase activity, using equal milk-coagulating quantities of these four enzyme preparations on hemoglobin, were obtained at pH values from 3.5 to 3.7. The proteinase activity of rennet extract on hemoglobin at pH values from 1.5 to 2.5 was considerably greater than that of the rennin. This indicated that pepsin present in rennet extract was partially or completely removed during the purification of rennin. These results also definitely indicated that rennin was not only a coagulating enzyme but possessed hydrolytic properties as well.

The action of these enzyme preparations on casein was studied and it was determined that the extent of proteolysis by rennet extract at pH 2.0 was approximately the same as it was at pH 5.4. This is additional evidence that rennet extract is composed of pepsin and rennin. The activity of rennin and the rennet powders was greater on casein at a pH of 5.4 than it was at pH 2.0. This showed that these enzymes had been freed of pepsin to some extent in their preparation. It also indicated that rennin was capable of proteolyzing casein at a pH value not far removed from that of ripening cheddar cheese.

A cell-free extract of *S. lactis* was prepared by growing the organisms in a broth medium. The acidity which developed was neutralized from time to time during the incubation period. The bacterial cells were separated from the medium using a Sharples supercentrifuge. A cell-free extract of the harvested cells was prepared by alternately freezing and thawing the cells and grinding them with powdered glass.

Two series of cheese were made in which cell-free extract was added to the milk used for manufacture of cheese. Cysteine hydrochloride, alone and in combination with the cell-free extract of *S. lactis*, was added in the manufacture of this cheese. The rate of ripening was not appreciably affected by the addition of these substances to the cheese.

Proteolytic activity associated with the cell-free extract of *S. lactis* was too slight to be measured accurately using the modified method of Anson.

Peptidase activity present in the cell-free extract of *S. lactis* was estimated using the method of Linderstrøm-Lang and employing glycyl-L-leucine and DL-alanylglycine as substrates. The hydrolysis of DL-alanylglycine proceeded at a much more rapid rate than did the hydrolysis of glycyl-L-leucine. A pH optimum at 7.6 to 7.8 was observed for the hydrolysis of glycyl-L-leucine. The optimum reaction for the hydrolysis of DL-alanylglycine by the cell-free extract of *S. lactis* was about pH 8.0.

The effect of the incubation of the cell-free extract of *S. lactis* in the presence of certain metallic ions on the hydrolysis of glycyl-L-leucine and DL-alanylglycine was studied. The manganese ion stimulated the action of the cell-free extract on glycyl-L-leucine. Copper, nickel, and zinc ions retarded hydrolysis of glycyl-L-leucine; magnesium was without appreciable effect. The hydrolysis of DL-alanylglycine was retarded by the presence of manganese ions, and copper, nickel, and zinc ions also retarded this reaction, but to a lesser degree than did manganese. The magnesium had no appreciable effect on the hydrolysis of DL-alanylglycine.

It was concluded that rennet extract was an important factor in the ripening process of cheddar cheese. However, rennet extract was not the critical factor in development of cheddar cheese flavor. When pasteurized milk cheddar cheese is made with 3 ounces of rennet extract per 1,000 pounds of milk, enzymes other than rennet extract contribute as much or more to the hydrolysis of the cheese protein as does rennet extract. These enzymes are considered to be largely of bacterial origin. The action of the endo-cellular proteolytic enzymes of *S. lactis* has been shown to be affected by very small quantities of metallic ions. This may help to explain the known effects of metals such as copper on the retardation of flavor development in cheddar cheese.

EFFECT OF THE Y-y FACTOR PAIR ON YIELD AND OTHER AGRONOMIC CHARACTERS IN CORN ¹

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Data available from variety comparisons in the southern part of the Corn Belt and the South indicate many white strains are superior in yield to the highest yielding yellow strains. The same general situation prevails with hybrids grown in this same area. Limited data indicated the higher yielding white endosperm types to be later in maturity. The question is often raised as to whether there is any necessary relationship between endosperm color and yield. An additional interest from a breeding standpoint was the possible relationship of endosperm color with other agronomic characters.

The method employed was to contrast the top-cross performance of Y and y segregates from white and yellow crosses. A midseason yellow inbred line WF9 and a late white inbred line Mo22 were chosen for parents of these crosses. The parents represented lines of high combining ability sufficiently different in maturity for their crosses to segregate for this character. Top-cross testers consisted of a white and a yellow open-pollinated variety to determine the presence of an interaction between endosperm color of the segregates with color of the testers.

Yield is generally assumed to be conditioned by environment and a large number of genes distributed at random over the chromosome complement. Therefore the separation of the Y and y segregates from an F₂ population could at best mark only a small segment of the sixth chromosome adjacent to the Y locus and would give little information on the gene frequency for factors conditioning yield.

The F₂ segregates consequently were supplemented by the use of the backcrosses to both parents. By this approach contrasts were available for Y and y segregates when the residual genetic background was made up of 25, 50, and 75 per cent of the original genotype of each parental line.

Seed from the F₂, BC₁, and BC₂ crosses were separated into Y and y classes. Plants from these crosses were selfed and outcrossed to the two top-cross testers.

The top-cross performance trials were conducted at the two locations Marshall and Jefferson City, Missouri in 1948. The mean yield for the two locations was 56.2 and 82.7 bushels respectively, indicating the relative productivity of the two fields.

¹ Doctoral thesis number 1033 submitted March 9, 1950.

The means for each tester by location were computed according to their genetic constitution F_2W , F_2Y , BC_1Y , BC_2W , and BC_2Y so that the means from the two experiments were subdivided into twenty groups. The within-group variance and corresponding degrees of freedom were calculated and these twenty estimates of variance were combined to yield a pooled variance for testing the significance of the various comparisons.

The comparisons of means were accomplished by setting up an orthogonal set of four contrasts consisting of α , β , γ , and δ . α contrasted the effects of the y segregates of the F_2W and BC_2W populations and the Y segregates of the F_2Y and the BC_2Y populations. β provided a contrast of the F_2 segregates against BC_2 segregates disregarding color. The fourth contrast, δ , represented comparison of the y segregates of F_2W and the Y segregates of BC_2Y against the Y segregate of the F_2Y and the y segregates of the BC_2W . The γ contrast was of little biological significance and was added only to complete the orthogonality of the set.

The α , β , γ , and δ contrasts among the Y and y segregates from the top-cross performance data revealed the following:

1. No association was found between yield and color of endosperm.
2. Moisture content of the grain at harvest was significantly higher for the white endosperm segregates. This suggested the white endosperm segregates on the average were later in maturity than the yellow segregates.
3. Ear height grades were significantly higher for the white endosperm segregates which also indicated later maturity for this group.
4. Significant positive correlations between yield and moisture indicated the later maturing segregates were producing higher yields.
5. Top-cross ear heights were significantly correlated with moisture as were the pollination dates of the S_0 plants with moisture of their corresponding top crosses. These data indicated that moisture was a suitable criterion of maturity.
6. Differences in husk cover grade between the two endosperm colors were not evident.
7. Although there appeared to be a rather good agreement of the yield performance of all segregates with the two testers, the Y segregates gave a more consistent performance between testers than the y segregates. Comparison of the yield performance of the F_2 , BC_1 , and BC_2 segregates between testers indicated that where a larger percentage of the genetic constitution was made up of white parentage the poorer was the agreement between testers.
8. Segregates with a proportionally higher percentage of their genetic constitution contributed by the white parent gave higher yields, moisture percentage and ear height grades at location 2. These same segregates did not exhibit this relationship at location 1.

From a study of individual plant measurements involving Y- and y-colored endosperm no significant differences were found for ear weight, number of days from planting to silking, or the number of ears per plant. A significant difference was found for ear height. Plants grown from the y classified seeds had a greater average ear height. The top-cross performance study indicated greater ear height grades denoted later maturity. Therefore, these data suggest factors for later maturity were linked with white endosperm color. This association is in general agreement with the results of the top-cross performance trials.

LIST OF MASTER'S THESES
FOR THE ACADEMIC YEAR 1949-50

- Agan, Raymond John. Swine management practices used by participants of the institutional on-farm training program for veterans.
- Ahmann, John Stanley. Effectiveness of recitation-laboratory and lecture-recitation-laboratory methods for teaching introductory chemistry to freshman students in engineering.
- Ali, Syed Aejaz. Design and performance of a vibrating cultivator.
- Amer, Fathi Mohamed. Influence of oxygen percentage and moisture tension on nitrification in soils.
- Anderson, Chester Robert. Occupational progress made by industrial arts students of Lincoln High School, Kansas City, Missouri.
- Anderson, John Ernest. Comparison of some methods for determining emulsion stability.
- Anderson, Russell Kenneth. Lactation effects from feeding alfalfa during gestation.
- Appell, Sam Darling. Industrial education in some of the colleges of Nebraska.
- Arkwright, Marjorie Starr. Equipment selection and layout designs for food service at Panahou School, Honolulu.
- Atkinson, Elsie Hemmingson. Syntheses, properties and applications of some tetrazolium compounds.
- Ayers, Augustus Sidney. Determination of trace quantities of iron in zirconium and its compounds.
- Bailey, Arthur Paul. Forecasting graduation probabilities for engineering students at the University of New Mexico.
- Bailey, Minnie Thomas. Relation of type of school to pupil achievement in the Negro elementary schools of Giles County, Tennessee.
- Bailey, Shady Olus. Construction and testing an ionization chamber for monitoring a synchrotron beam.
- Balster, Clifford Arthur. Surface geology of Calhoun County.
- Baringer, Maurice Edmund. Trace minerals for swine.
- Barnett, Doris Marie. Relative merits of fresh whole egg and dried whole egg in sponge cake for institution food service.
- Baumgartner, Ruth Elizabeth. Factors related to enrollment and persistence of attendance in adult homemaking classes.
- Baxter, Reginald Robert. Pyrophosphate copper plating baths.
- Bayles, Charles Courtney. Effect of different sources of vitamin B₁₂ on hatchability of eggs and early chick growth.
- Bear, Janet Elizabeth. Formulation of a partially synthetic ration adequate for reproduction of rats.
- Bednar, Ernest George. Professional preparation and instructional duties of Montana industrial arts teachers.

- Bell, Everette Lyle. Factors influencing occupational choices of men qualified at Iowa State College since 1939 to teach Vocational Agriculture.
- Benedict, Howard Norman. Cleavage reactions of some unsymmetrical disiloxanes.
- Bennett, William George. The life history of *Leucopis simplex* Loew, (Diptera Chamaemyiidae).
- Bittner, Frederick J. Transverse area assumed uniformly stressed in the threaded section of stud bolts loaded in tension.
- Blinn, Edmund George. Some Iowa weekly newspaper conditions which influence employment decisions of college journalism graduates.
- Bolie, Victor. Pull-out torque of a synchronous motor.
- Bowles, Robert Lewis. Accuracy of certain approximate methods in predicting the correct model for experiments with unequal frequencies in the subclasses.
- Britton, Charles Cooper. Analysis of electric circuit transients by Taylor's series.
- Britton, Maxine Harp. Nutritional status and dietary requirements of older women. IV. Evaluation of vitamin C status of 50 women.
- Broschat, Richard Ernest. Effects of cold working on flexural hysteresis of sheet phosphor bronze.
- Brown, Ray Alexander. An analysis of the roadable airplane.
- Brubaker, Joseph Junior. Water treatment by means of a fine-grain filter media.
- Brule, John Dosithe. Analysis of wound rotor induction motors for synchronized drives.
- Bryan, Ashley Monroe. Absorption of 2,4-dichlorophenoxyacetic acid by leaves.
- Buck, Griffith J. Root behavior in fall and spring planted roses.
- Bul, Auke Alexander. Some design factors in the redevelopment of blighted urban residential areas.
- Bulls, John Thomas. History of extension education in agriculture and home economics among Negroes in Alabama.
- Burnet, George, Jr. Effect of pressure on the settling characteristics of suspensions.
- Carneiro, Geraldo Goncalves. Reproductive rates and growth of purebred Schwyz cattle in Brazil.
- Carter, Gordon Robert. Immunizing value of a *Pasteurella Multocida* chicken embryo vaccine.
- Carvey, Robert Morse. Various chlorinated solvents for the extraction of soybean oil.
- Chappell, William Martin. Biharmonic problems satisfying Navier's boundary conditions.
- Chen, Fang Lu. Water requirement of swine.
- Chen, Hsi Hsiung. Inheritance of self-fertility in Autotetraploid sweet clover, *Melilotus alba*.
- Chen, Shao-Li. Utilization of the water-soluble substances from trichloroethylene-extracted soybean meal.

- Chia, Za-sung. A study of thin film lubrication.
- Chiang, Shu-nan. Longicorn beetles of Kwangsi and Kweichow provinces of China.
- Christensen, Herluf Wilmer. A rating of rural community schools in Iowa.
- Christensen, Isabel Loraine. Nutritional status and dietary requirements of older women. III. The basal metabolism of forty-seven adult women ranging in age from 32 to 76 years.
- Cleal, Harold Leslie. Methods used to promote certified seed with analysis of their costs.
- Clingman, Wilbur Dean. Elastic curve of a simple beam under impact loading.
- Cochrane, Robert Ernest. Effects of substituting sound motion pictures for laboratory practice upon achievement in high school mechanical drawing.
- Comerford, Sister Mary Maurita. Nutritional status and dietary requirements of older women. I. Ascorbic acid.
- Connolly, Sylvia Pedersen. Attitudes toward small children reported by girls enrolled in vocational home economics departments in Iowa.
- Conrad, Karl LeRoy. Stress distribution due to hydrostatic pressure on a parabolic boundary.
- Cosby, Clifford Wayne. Weighting of characteristics of high school graduates for determining probability of entrance to college.
- Coulter, Naomi Gilkey. Uses made of training in trade dressmaking at Dunbar High School, Little Rock, Arkansas.
- Cunningham, Gerald Ashley. Services offered in small communities in Central Iowa whose high schools were closed between 1939-1949.
- Dana, Malcolm Niven. Response of apple scion buds to treatments for breaking rest period.
- Day, Grace Hardy. Concentration of anion for the mordanting of wool fiber in dichromate.
- DeKoster, Roger Paul. Certification of industrial arts teachers in the United States.
- Deter, Eugene Edward. Determination and consolidation of management concepts of normal productive effort in a small manufacturing concern.
- DeVore, James A. Provisions for teaching industrial arts in seven Iowa counties.
- Dixon, Joseph Harold. Influence lines for the design of two-hinged arches.
- Dodds, Barbara Degen. Relative effectiveness of paired statements and single statements in a scale designed to measure attitudes toward education.
- Dolnick, Irving Charles. Probable effects of various locational policies on the city of Ames.
- Dominy, Thama Faye. Cost distribution and nutritive content of food served at Mary Lyon Hall, Iowa State College.

- Donahoo, Alvin William. Soil management practices used by participants in the institutional on-farm training program for veterans.
- Donovan, Austin Oliver. American economic policy and administration in the Virgin Islands, 1917-1950.
- Dorheim, Fred Houge. Petrography of selected limestone aggregates.
- Dorsett, George Lehner. Certain problems of a group of wood-using industries in Iowa.
- Dove, Richard Charles. Determination of the effective strained length of standard stud bolts.
- Dow, William Andrew. Investigation of theory of pounding in direct mixing of steam and water.
- Dreste, Fred Edwin. An electronic illumination indicator for the blind.
- Dunigan, Maryann. Test for measuring the ability of parents to apply selected generalizations in child development.
- Dutz, Hans Georg. Flow of ponded water into tile drains as affected by space between individual tiles.
- Ebersole, Nancy Roberta. Nutritional status of Iowa children. II. Concentration of hemoglobin in blood of children attending schools with and without school lunch programs.
- Eggert, Myers Robert. Status of Missouri industrial arts instructors.
- Einspahr, Dean William. Site index of oak in relation to soil and topography.
- El-Attar, Abel Hamid Zawzi. Future price policy for cotton in the United States.
- Elderkin, Frank Joel. Alfalfa-bromegrass mixture as affected by variety and proportion of legume and grass in the seeding.
- Elderkin, John Dale. Risk and uncertainty in crop production.
- Empey, Gene Francis. Reader preference in a magazine-type publication presenting farm and home information.
- Englebrecht, Roger Evan. Usefulness of the Kuder preference record for predicting achievement in woodworking at the Iowa State College.
- Esser, Frank Robert. Essentials of a production planning and control system for a small- or medium-sized plant.
- Eusebio, Alfonso Napalang. Influence of coliform bacteria on growth of lactic streptococci.
- Evans, Daniel Donald. Measurement of the air permeability of soil *in situ*.
- Fackler, Jean Georgia. Dating problems of high school boys and girls in towns with populations of 4,000 to 10,000 in Iowa.
- Farrier, Maurice H. Hard pine cone-worm (*Dioryctria Auranticella* Grote, Phycitidae) and its parasites in Iowa.
- Ferguson, Raymond Craig. Spectrophotometric determination of nickel with vic-dioximes.
- Fitch, William Chester. Fundamental aspects of depreciation theory.
- Fleming, Rodney Rae. Urban pavements: deterioration and repair.
- Fontes, Luiz Rodrigues. Differences in growth and live weight among Zebu cattle breeds in Brazil.

- Forker, Barbara Ellen. Skill levels in women's physical education at Iowa State College.
- Forman, Wallace Rex. Effects of the fair-trade acts on retail prices, 1939-1950.
- Fox, Onis Venita. Relation between assertive behavior and social acceptance in nursery school children.
- Frazer, John Ronald. A study of creamery operations from an industrial engineering viewpoint.
- Freeman, Ralph Leslie. Nature and significance of residual stresses in heat-treated steel.
- French, Burton Leroy. Application of simultaneous equations to the analysis of the demand for meat.
- Fricke, Emmett Walter. Pupil interest in industrial arts subjects in the Sioux City Junior High Schools.
- Frunk, William Don. Low temperature effects on insect fumigants.
- Fuller, Dorothy Craft. Amount and type of authority exercised by parents of preschool children as related to authority in parental homes.
- Fuller, George Milton. Wind tunnel tests of three wing tip ailerons in the presence of a vertical tip fin.
- Fung, Sui-tong Chan. Developmental effects of a sex gene in *Drosophila melanogaster*.
- Galley, Cyrus Abel. Effectiveness of teaching basic elements of industrial arts as an introductory seventh grade course.
- Gamble, William Keith. Effectiveness of vocational agriculture as preparation for a college course in poultry husbandry.
- Gardner, Eugene Vernon. Undergraduate offerings in industrial education.
- Gates, Leslie Dean, Jr. Temperatures in a plastic bushing under oscillatory loads.
- Geiger, Harlan Ervan. Opinions of farm young men and women in Keokuk County with reference to an extension program for young people.
- Geise, Charles Edward. Moisture studies of sweet corn varieties: I. Changes within the range of canning maturity. II. Comparison of methods of determination.
- Gertel, Karl. Benefits and costs of land improvements: a case study of the Nepper watershed in western Iowa.
- Gilkey, Herbert Talbot. Incremental control of a continuous input gas burner in a domestic furnace.
- Gilliam, Ezra Coke. Factors influencing the successful establishment and utilization of birdsfoot trefoil.
- Gonzalez-Chapel, Antonio. Comparative performance of the native Puerto Rican fowl, the White Leghorn, the New Hampshire and crosses between them.
- Gosslee, David Gilbert. Statistical analysis of weekly maximum temperature series for Mason City, Iowa.

- Graham, Frederick Mitchell. Analysis of continuous frames by distribution of deformation.
- Grandjean, William Burke. Pressure drop studies on a liquid-liquid extraction spray column.
- Gray, Basil Dale. Some reasons why dropouts left secondary schools in Marshall County, Iowa, 1944-1949.
- Gray, Eleanor Mary. Influence of certain factors on baking practices of Ames homemakers.
- Grecn, Sister Margaret Dolores. The development of Roman Catholic Church vestments.
- Gruenwald, Ralph Wirth. Cropping practices used by veterans enrolled in institutional on-farm training program.
- Guenther, Edwin Lynn. Performance of seedling progenies of *Bromus inermis*, with different methods of planting, as related to the yield of parental clones.
- Guttay, John Robert. Germination, composition and yield of plants as affected by seed treatment with fertilizer salts.
- Gwinn, Aral Boyd. Effects of vine killing practices on yield, specific gravity and internal tuber discoloration in the Irish potato.
- Haas, Mabel Nissen. Adult education activities of the woman's Societies of Christian Service of Boone district.
- Hall, Ruth Eleanor. Certain factors related to the clothing problems of freshman women at Iowa State College.
- Hallsted, Margaret Prue. Problems in personal-social relationships recognized by high school girls and boys in three similar Illinois towns.
- Hamlin, Charles Everlin. Relationship between size and strength of glued joints.
- Hansen, Jean McDirmid. Reasons given for non-participation in adult homemaking classes in Beardshear district, Ames, Iowa.
- Hanson, Durwin Melford. Apprenticeship and on-the-job training programs in Iowa.
- Hardy, John Lawrence. Toxicity of chemicals to *Venturia inaequalis* (Cke.) Wint.
- Harris, James Cleo. Characteristics of Iowa's longest school bus routes.
- Haskett, William Courtney. Relative susceptibility of barley varieties to *Pythium graminicola* Subr.
- Hegel, Roberto Solis. Experimental determination of web and flexural strains in an I-shaped beam of prestressed concrete.
- Heidemann, Leonard W. Acceptance of the English language in the Lutheran Church, Missouri Synod.
- Heimann, Henry Carl. Vegetative reproduction of reed canary grass (*Phalaris arundinacea*).
- Heiman, Warren Jonas. Fast neutron bombardment of germanium.
- Held, Hallie LaVonne. Regional characteristics reflected in a designer's work.
- Held, Royer Burnell. Problems and criteria relating to action programs on private agricultural land.

- Helmstadter, Gerald Carl. Relation of intellectual trait differences to intellectual plasticity.
- Henderson, Harry Danner. Facilities and equipment for vocational agriculture in Iowa.
- Henry, Lyell Dewel. Measurement of effect of composition of work cycle upon difficulty and pace of an operation.
- Herdman, Raymond Wain. Predicting pupil mortality among high school boys.
- Hermesmeier, Lee F. Terraces constructed with five types of machines in western Iowa.
- Herrick, John Berne. Cytological changes in the cervical mucosa of the cow (*Bos Taurus*) throughout the estrous cycle.
- Hildreth, Roland James, Jr. Collective bargaining in the meat packing industry.
- Hill, Ivan Leroy. Predicting achievement in descriptive geometry.
- Hiltbold, Arthur Edward. Rate of biological interchange between fertilizer and soil organic nitrogen as influenced by crops and addition of plant residues.
- Hiltbold, Helen Marian Thompson. Performance of commonly used seam finishes on certain cotton, linen and nylon fabrics.
- Hoffman, Paul Fredrick, Jr. Chemotherapy and spread of oak wilt.
- Holman, Elizabeth Jeanne. Benzene complexes of silver perchlorate and tetramethylplatinum.
- Holman, Iletta Marcella. Art and certain aspects of personality.
- Hoppe, Donald Alvin. Perception of longitudinal speed differentials between vehicles on the highway at night.
- Hosmer, William T. A system of accounts and accounting procedures for county highway departments in Iowa.
- Houdyshell, Marie. Problems of family life recommended for study in high school and junior college by graduates of Marshalltown High School.
- Houston, Kenneth Leverett. Occupations of farm-reared boys who were graduated from high schools offering vocational agriculture.
- Hsia, Pong Ray. Development of solvent extraction equipment for oil-bearing seeds.
- Hughes, Arden Boyd. Accumulation of ingested DDT in gonads and adrenal glands of white rat, and associated changes in cholesterol content of the ovaries.
- Hull, Elizabeth Katherine. Problems in dating recognized by ninth and tenth grade girls in two population groups.
- Hunter, Ray. Physical properties of some loess-derived prairie soils of southeastern Iowa.
- Hunzeker, Hubert LaVon. Centers of forces of attraction for certain geometrical bodies.
- Hunziker, Ruppert Rudolph. Phosphorus compounds in oats and alfalfa as affected by phosphorus fertilization.
- Hurry, Harry Frederick. Factors affecting feather and body growth in chickens.

- Husain, Sheikh Mohammad Aijaz. Cost relationships in farm machinery use.
- Hutchcroft, Charles Dennett. Accuracy of estimating the mean percentage of non-detasseled plants in double cross corn seed production fields.
- Inman, Lydia Lucille. Effect of packaging and judging procedures on scores of ground pork and beef stored at fluctuating temperatures in a home freezer.
- Isaac, Eugene Leonard. Status of Mississippi and South Carolina Negro day trade teachers in 1949.
- Jackson, Robert Willard. History of corn harvesting machinery.
- Jensen, Lloyd Everett. Analysis of an eddy current brake.
- Johnson, Betty Virginia. School lunch training programs for cook-managers.
- Johnson, Maud Esther. Relation of extension service goals for family food production and storage to practices and facilities of Negro farm families in Houston County, Texas.
- Jones, Grant Calvin. Work permits and street trades permits in Iowa, 1949.
- Jones, Hayden Halsey, Jr. Some uses for synthetic siliceous zeolite fines.
- Jones, Jacqueline Marie. Variations in the biological activity of cytochrome C preparations.
- Joos, Lloyd Leland. Availability of rock phosphate fertilizer as affected by particle size, incubation and soil reaction.
- Judge, George Garrett. Determinants of the extent and type of cattle feeding in Iowa.
- Juvonen, Lea Matilda Orvokki. Food and house service in some logging camps in the United States and Finland.
- Kam, Ah Leong. Hawaiian aggregates in bituminous concrete.
- Kemp, John Bernard. Application of economic analysis to determine measures for flood prevention and control.
- Ketcham, George Gardiner. Spectrographic determination of gadolinium and samarium in purified yttrium.
- Kinsey, Roy Henry. Synthesis of new organic analytical reagents.
- Kirkpatrick, Herman Howard. Teaching of physics in secondary schools of Iowa.
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